An evaluation of the performance and usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo South Africa

Devanand Moonasar

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Disease Control and Vector Biology Unit

Department of Infectious and Tropical Diseases

London School of Hygiene and Tropical Medicine

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This thesis is dedicated to my sister

Shireen, whose inspiring words, echo in my mind, long after her passing.....

Abstract:

Aim: This thesis aimed to evaluate the performance and usage of ICT Pf Malaria Rapid Diagnostic Test (MRDT), in an operational setting in the Limpopo Province, South Africa.

Methods: Four studies were conducted to: assess factors affecting MRDT use (exploratory study- conducted as part of formative work); determine ICT Pf accuracy (cross-sectional study amongst 405 patients with prospective observational cohort component for follow-up); determine the performance of MRDT end-users (cross-sectional observational study) and assess the suitability of using positive control antigen wells (PCWs) for routine quality control.

Results: Key informants reported that MRDT accuracy, end-user proficiency and MRDT quality affect MRDT use and impact. The accuracy study found that sensitivity, specificity, positive and negative predictive values of ICT Pf test were 99.48% (99% CI; 96.17-100.00%), 96.26% (99% CI; 94.7-100%) 98.48 (99% CI 98.41 -100.00%) and 96.26% (99% CI 91.53-98.79%) respectively. Febrile patients with 'sweating' were 5 times more likely to be ICT Pf positive than those without sweating. Among the 68 patients who returned for day-seven follow up 23 (33%) were ICT Pf positive; however all were microscopy-negative. End-user proficiency: of the 15 recommended steps for MRDT use, 50% of end-users performed 11 or more steps correctly; 50% of end-users interpreted 90% of pre-prepared tests correctly. The false negative interpretation rate was 15%. The quality control study revealed that diluting PCWs with MRDT-negative blood gave better signals than diluting with citrate buffer. PCWs maintain signal strength when stored up to 30 days at 25^0C at rural health clinics.

Conclusions: Although ICT Pf MRDT can be used for malaria diagnosis in Limpopo, test sensitivity at low level parasitaemias in field settings need to be established. The ICT Pf test should not be used for assessing cure post-treatment. End-user proficiency needs improvement. PCWs can be used to monitor MRDT quality at PHC level.

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I would like to acknowledge the following persons for providing support to me so that this work could reach fruition.

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Glossary of Terms

Antigenaemia	Parasite specific antigens present in malaria infected blood.
Combination therapy	Artemisinin based therapy, using 2 drugs co-artemether and Lumefantrine (in the study context).
Diagnostic accuracy	The test accuracy as measured by sensitivity and specificity.
End user	Health workers performing MRDTs.
False positive rate:	The proportion of individuals without infection being missed by the test and falsely ascribed a positive status.
False Negative rate:	The proportion of the infected individuals being missed by the test and falsely ascribed a negative status.
Gold Standard	A method or procedure that is widely accepted and recognized, microscopy was the gold standard for diagnosis in this study.
Health workers	Persons working at health facilities; e.g. nurses and nursing assistants.
J index	Overall measure of the reliability of a diagnostic test which summaries both sensitivity and specificity.

Low transmission setting	Malaria cases, less than 10 per 1000 population at risk.
LRT test	Likelihood Ratio Test - The likelihood ratio incorporates both the sensitivity and specificity of the test and provides a direct estimate of how much a test result will change the odds of having a disease. The likelihood ratio for a positive result (LR+) tells you how much the odds of the disease increase when a test is positive. The likelihood ratio for a negative result (LR-) tells you how much the odds of the disease decrease when a test is negative.
Managers	Officials responsible for co-ordinating programmatic activities, in this study context the malaria programme managers at district level were responsible for coordinating malaria operations at the implementation level.
Monotherapy	Antimalarial treatment with a single medicine.
Negative predictive value	The proportion of the test's negative reading which is true.
Overdiagnosis	False positive diagnosis, when diagnosis takes place incorrectly sometimes on a clinical basis rather than through definitive diagnosis (laboratory or rapid test).
Parasitaemia	When malaria parasites are present in the blood stream.
Periurban	Areas that fall on the periphery of urban settings.

Positive Predictive value	Is the proportion of a test's positive reading that is truly positive.
Predictors of malaria	Clinical symptoms that raise suspicion for diagnosis of malaria.
Sensitivity	The ability of a test to detect infected individuals as positive.
Specificity	The ability of a test to detect individuals without an infection as negative.
Suspected malaria	Patients presenting with fever, headache and / or chills and sweating.
Unresolved malaria	This included patients who on follow-up had symptoms of fever chills, sweating, headache, nausea or vomiting.

List of Abbreviations

CI	Confidence Interval
DM	Devanand Moonasar
ELISA	Enzyme-linked immuno-assay
Fig	Figure
HRP(II)	Histidine Rich Protein (II)
ICT	Immuno-chromatographic Test/s
ICT Pf	Immuno-chromatographic test *Plasmodium falciparum*
LDH	Lactate Dehydrogenase
LRT	Likelihood Ratio Test
MRDTs	Malaria Rapid Diagnostic Tests
MRDDS	Malaria Rapid Diagnostic Devices
NPV	Negative Predictive Values
PCR	Polymerase chain reaction
PCW	Positive Control Wells
Pf	*Plasmodium falciparum*
PPV	Positive Predictive Values
PLDH	Parasite Lactate Dehyrogenase
PHC	Primary Health Care Clinics
UK	United Kingdom
VDM	Vhembe District Municipality
WHO	World Health Organisation

1.0 Chapter 1: Introduction

The prompt and accurate diagnosis of malaria is crucial to prevent malaria-related complications and mortality. In South Africa, in most primary and secondary health facilities, Malaria Rapid Diagnostic Tests (MRDTs) are used to make a definitive diagnosis of malaria. The national malaria treatment guidelines recommend giving anti-malaria treatment to MRDT positive cases only. Thus the performance and use of MRDTs play an important role to prevent malaria-related complications and mortality in South Africa. This study aimed to evaluate the performance and usage of ICT Pf Malaria Rapid Diagnostic Test (MRDT), in an operational setting in the Limpopo Province, South Africa. Specifically it aimed to assess factors affecting MRDT use, determine the accuracy of MRDTs in the field, assess the performance of MRDT end-users (cross-sectional observational study) and assess the reliability of using positive control antigen wells for routine quality control of MRDTs in the field.

Chapter 2 presents the background to the study: It provides a comprehensive review of the literature on MRDT accuracy, end-user proficiency and quality control of MRDTs as well as the aims and objectives of the study, the problem statement, and the epidemiology and control of malaria in South Africa, with emphasis on the study area.

In chapter 3, the results of an exploratory study that was undertaken as formative work to identify factors that affect the performance and use of MRDTs at primary health care level in South Africa, are presented. The key findings from the exploratory study were that the accuracy of MRDTs, end-user proficiency and the quality of the MRDT test kit, were major factors that affect the usage and impact of MRDTs.

Acknowledging the results from the formative work (presented in chapter 3) the main study therefore focused on 3 key aspects: (1) accuracy of the MRDT, (2) end-user

proficiency and (3) use of positive control wells to assess the quality of the MRDTs in the field.

Chapter 4 presents the results of the study that assessed the accuracy of MRDTs.
Chapter 5 presents the results of the study that assessed MRDT end-user proficiency.
Chapter 6 reports the use of positive control wells for routine quality control of MRDTs.
Chapter 7 summarises and discusses the key findings of this work.

The appendices present more details on procedures and tools used for the study.

2.0 Chapter 2: Background

2.1 Statement of the Problem

The 2005 World Malaria Report estimates around 350-500 million clinical episodes of malaria and one million deaths due to malaria occur annually and that around 60% of the clinical cases and 80% of malaria deaths occur in Africa.(1) Malaria contributes to anaemia in children and pregnant women and to adverse pregnancy outcomes such as abortion, still births, and low birth weights. Furthermore it is well documented that malaria hampers economic growth and development.(2)

Ten percent of South Africa's population (approximately 4.4 million people) live in malaria endemic areas. The transmission of malaria is seasonal and epidemic prone in South Africa.(3) Malaria epidemics can cause large numbers of infections and deaths in a relatively short period of time.(4) Diagnosing malaria accurately and promptly is therefore paramount to preventing malaria-related morbidity and mortality.(5)

Malaria Rapid Diagnostic tests (MRDTs) are a fast and easy way to diagnose malaria particularly in the peripheral areas where there is limited access to skilled laboratory personnel.(6) Since 2003 MRDTs are being used for the diagnosis of malaria at primary health care level in the Limpopo Province, South Africa.(3) However the field accuracy of MRDTs and end-user proficiency in MRDT use, are unknown.(7) Furthermore there is no system in the Limpopo Province to monitor the quality of MRDTs after the test has been exposed to field conditions.

The South African malaria treatment policy stipulates that all patients with a positive MRDT should receive anti-malarial treatment and that antimalarial drugs should be given only to those patients who have a definitive diagnosis of malaria reached through MRDT or microscopy.(8) This latter aspect of this policy raises concerns, given the possible limitations of MRDTs and especially the fact that MRDTs can give false negative results.(9) It is therefore important to estimate the accuracy of MRDTs

under field conditions. It is also important to understand the factors that affect the accuracy and use of MRDTs and to determine how to set up a quality control system to ensure appropriate use of MRDTs in South Africa.

2.2 Literature review

2.2.1 The Malaria profile in South Africa

Malaria transmission in South Africa can be defined as low to moderate and seasonal. The transmission of malaria occurs mainly during the rainy season from October to May and three out of nine provinces are affected by malaria.(3) The Limpopo Province, situated in the north-eastern part of South Africa, is one the three provinces where malaria transmission occurs, (Figure 1). From 2003 - 2006 the Limpopo has had higher numbers of reported malaria cases compared to the other two malaria-affected provinces (Figure 2). Malaria transmission decreased from about 41 786 cases in Kwa-Zulu Natal in the year 2000 to 2042 cases in the year 2003. This decrease can be attributed to change in drug policy - from monotherapy to ACTs; change in insecticide policy - from pyrethroids to DDT (Dichloro-Diphenhyl-Trichloroethane); establishing a cross-border malaria collaboration between Kwa-Zulu in South Africa, Maputo Province in Mozambique and Lubombo District in Swaziland.(3)

During the past five years (2001-2006), the Vhembe district, followed by the Mopani district has had on average, the highest number of malaria cases in the Limpopo province (Figure 3).(10) In a review of malaria data from the Limpopo province between 1999 to 2006, Gerritsen et al. states that the incidence of malaria in the Vhembe district is approximately 328.2 per 100 000 population at risk; more males than females are at risk of contracting malaria - the mean incidence of malaria was higher in males than females (145.8 vs 105.6 per 100 000 population at risk; $p<0.001$).(11) Their findings also point to malaria incidence being the lowest in the 0-4 year olds, peaking in the 35-39 age groups.

2.2.2 Demographics of the Vhembe District

The Vhembe District is one of Limpopo's six district municipalities established in 2000. It incorporates four local municipalities, namely: Makhado, Thulamela, Musina and Mutale. The Vhembe District Municipality (VDM) is situated in the northern part of Limpopo Province and is bordered to its south, east and west mainly by Central, Mopani and Botshabelo district municipalities; to its north lies Zimbabwe (Figure 4).

The Vhembe district covers 21 407 square kilometers of land and has a population over 1.1 million. The major languages spoken in Vhembe district are Xitsonga, Tshivenda, Sepedi, English and Afrikaans. Vhembe has a competitive advantage in agriculture, tourism and mining potentials, compared with the other Limpopo province districts. Malaria can affect income generation in Vhembe District, as all the potential economic development sectors, viz. agriculture, tourism and mining can be adversely affected by an increase in malaria.(2)

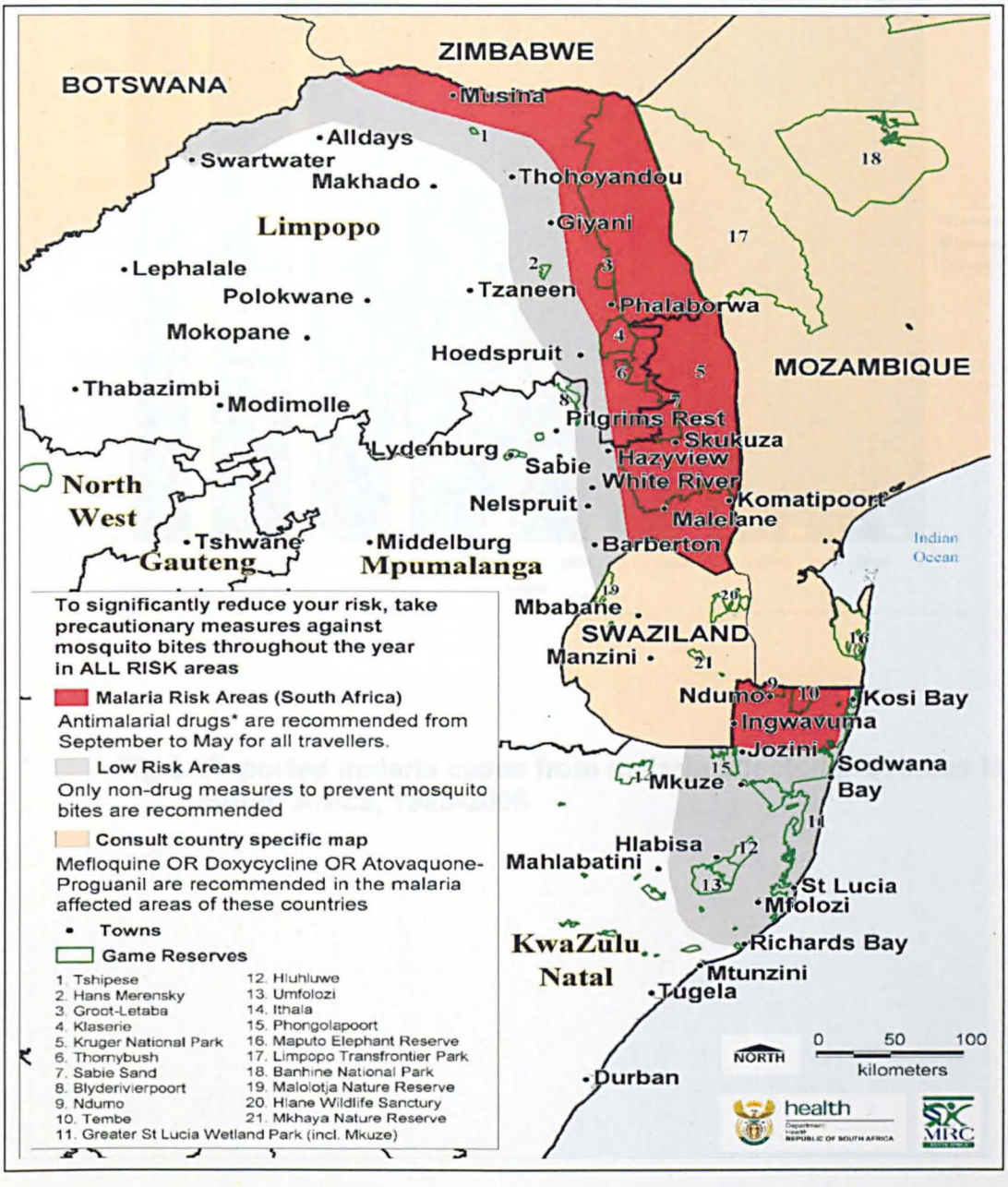


Fig 1: Malaria transmission risk areas of South Africa
(Source: National Department of Health- South Africa)

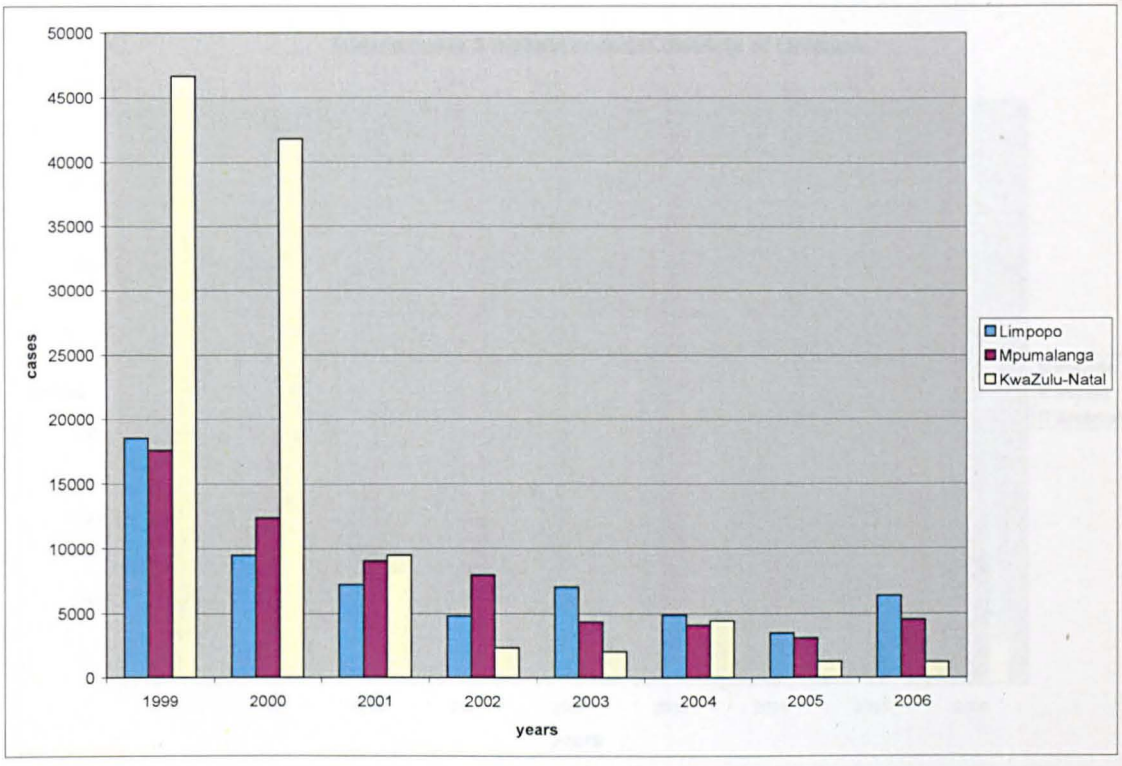


Fig 2: Reported malaria cases from malaria affected provinces in South Africa, 1999-2006

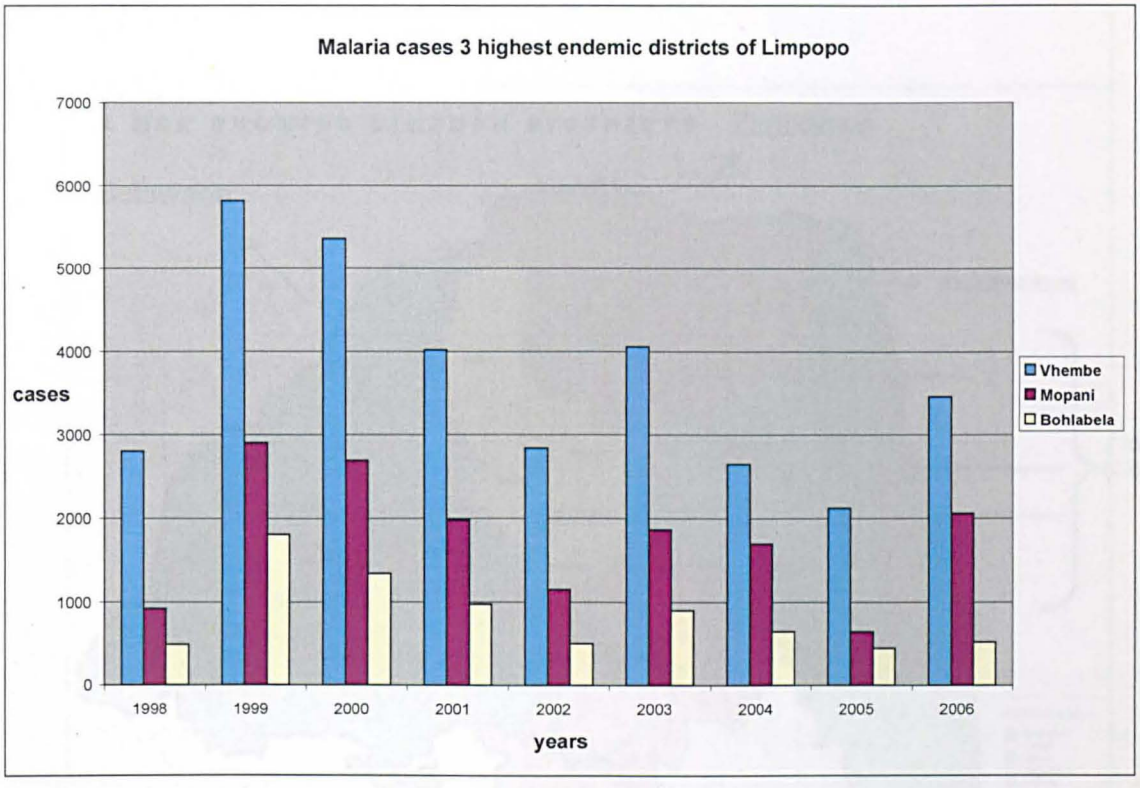
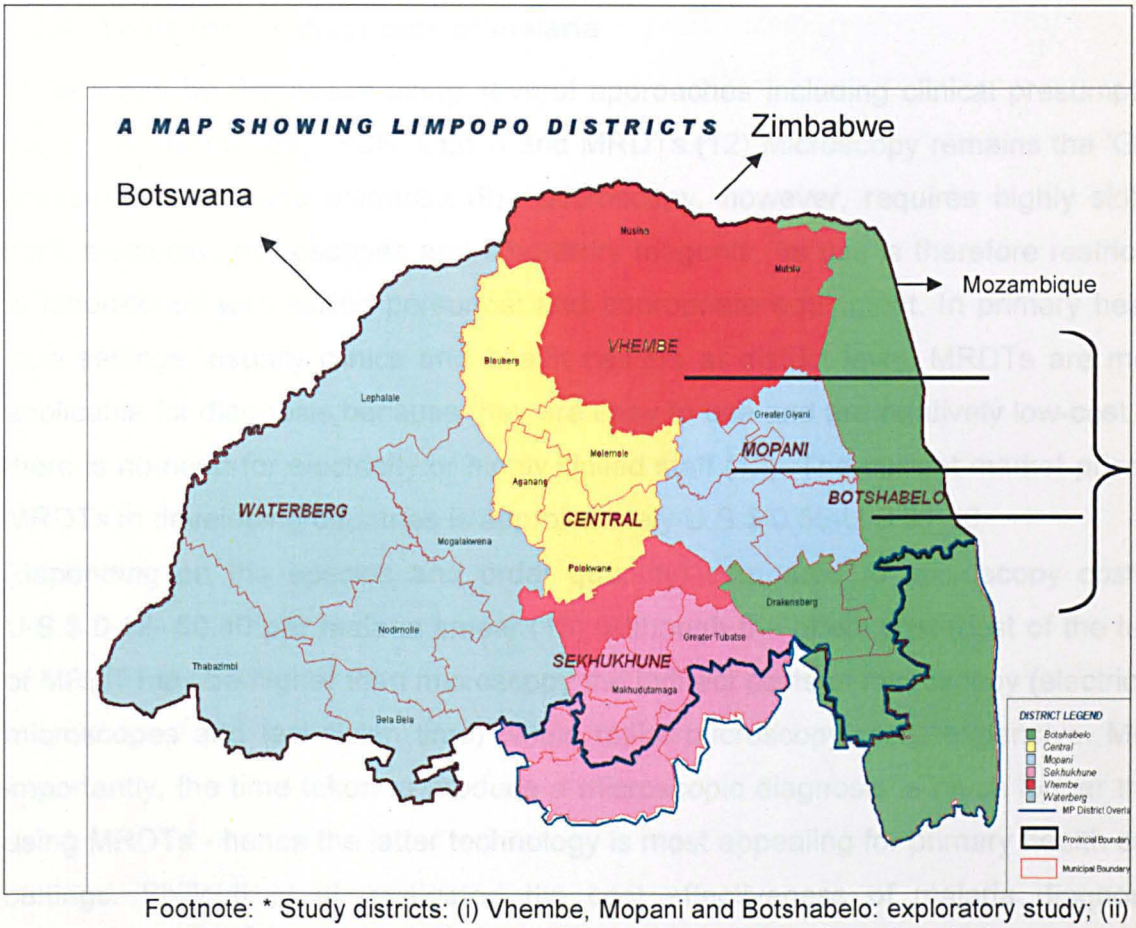


Fig 3: Comparison of malaria cases among high reporting districts in the Limpopo Province 1998-2006



Footnote: * Study districts: (i) Vhembe, Mopani and Botshabelo: exploratory study; (ii) Vhembe: accuracy; end-user proficiency study and quality control study

Fig 4 Districts of the Limpopo Province

(Source: Limpopo Department of Health and Social Welfare)

2.2.3 Strategies for controlling malaria

The World Health Organization's Global Malaria Strategy stipulates that early detection of cases or epidemics, rapid response to malaria epidemics and effective case management are key strategies for controlling malaria.(5) Definitive diagnosis using appropriate diagnostic tools is important to ensure early detection of malaria cases, ensure effective case management, prevent epidemics and reduce malaria-related mortality.(4-6)

2.2.4 Tools for the diagnosis of malaria

Malaria can be diagnosed using several approaches including clinical presumptive diagnosis, microscopy, PCR, ELISA and MRDTs.(12) Microscopy remains the 'Gold Standard' for malaria diagnosis.(9) Microscopy, however, requires highly skilled staff, electricity, microscopes and laboratory reagents. Its use is therefore restricted to laboratories with skilled personnel and appropriate equipment. In primary health care settings, usually clinics and health centers at district level, MRDTs are more applicable for diagnosis because they are easy to use and are relatively low-cost, as there is no need for electricity or highly skilled staff.(12) The current market price of MRDTs in developing countries is approximately U.S.$ 0.55-U.S.$1.50 (depending on the species and order quantity) compared to microscopy cost of U.S.$ 0.12- $0.40 per malaria smear.(13). Although the direct cost (cost of the test) of MRDT may be higher than microscopy the indirect costs of microscopy (electricity, microscopes and technician time) would make microscopy more expensive. More importantly, the time taken to produce a microscopic diagnosis is much longer than using MRDTs - hence the latter technology is most appealing for primary health care settings. Shillcutt et al. evaluated the cost effectiveness of malaria diagnostic methods in sub-Saharan Africa in an era of combination therapy.(14) The objective of their study was to estimate the relative cost-effectiveness of MRDTs, presumptive treatment and field standard microscopy in different epidemiological settings of sub-Saharan Africa where *Plasmodium falciparum* predominates, using a decision-tree model and probabilistic sensitivity analysis. Their findings revealed that MRDTs were cost-effective compared with presumptive treatment and microscopy.

2.2.5 Malaria diagnosis and treatment in South Africa

In South Africa the health-care system is decentralised, whereby policy is set at the national level and implementation takes place at the district level. In the district health-care system, commodities such as MRDTs are purchased through the district health budgets and distributed to the clinics via the district pharmacy stores. At the primary health-care level the first point of call for patients is the clinic, clinics refer severely ill patients to district hospitals.

The Limpopo Province, in keeping with the National Department of Health's malaria policy, has adopted appropriate case management (definitive diagnosis and prompt treatment) as one of its key strategies for controlling malaria.(3) In 2001, South Africa changed its drug policy from monotherapy to Artemesinin Combination Treatment (ACT), this warranted the need for definitive diagnosis.(3) MRDTs was chosen as the routine diagnostic tool, especially at primary health care settings throughout the malaria affected provinces, including Limpopo. MRDTs have therefore become an important diagnostic tool for the management of malaria, especially in remote areas of South Africa.

2.2.6 Consequences of inaccurate diagnosis of malaria

The misdiagnosis of malaria can lead to medical, social, and economic consequences.(15) Medical consequences of misdiagnosis at the individual level, include inappropriate treatment resulting in prolonged illness. Social consequences could include loss of faith in health care services and delayed care-seeking. Economic consequences could include loss of earnings or increased expenditure on transport, drugs and consultations.

The consequences of over-diagnosis of malaria could result in higher mortality from diseases other than malaria. Reyburn and colleagues demonstrated in a study from Tanzania that clinical diagnosis of malaria yielded more false positives than diagnosis using microscopy.(16) Over-diagnosis of malaria can lead to overuse of antimalarial drugs, and may even prevent diagnosis and treatment of other diseases.(15, 17-19) On the other hand, the under-diagnosis of malaria or false

negatives could result in lack of treatment of patients and can lead to malaria-related complications and even death.(6, 20, 21)

Ensuring diagnostic accuracy of MRDTs is therefore essential to preventing the unnecessary and avoidable consequences of over- or under- diagnosis of malaria.

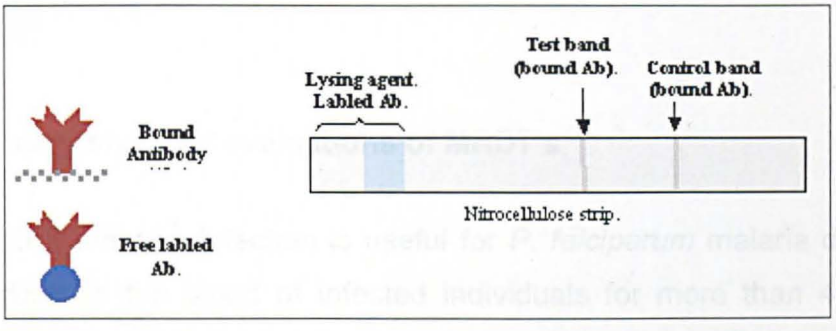
2.3 MRDTs for the diagnosis of malaria

2.3.1 Background to MRDTs

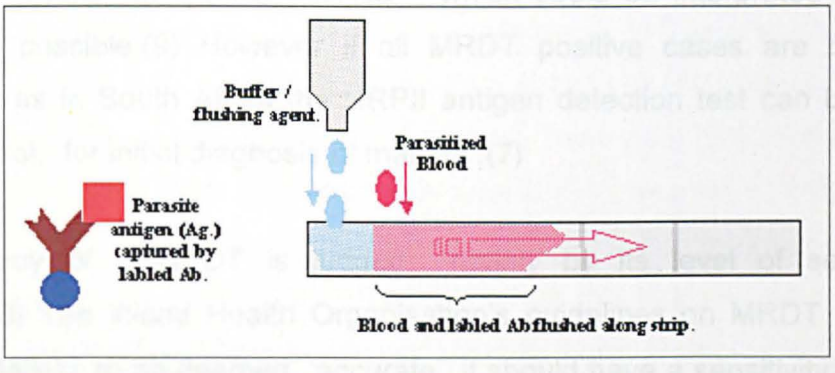
MRDTs, sometimes called Dipsticks or "Malaria Rapid Diagnostic Devices" (MRDDS), detect specific antigens (proteins), produced by malaria parasites.(6) There are currently 3 malaria parasite target antigens that can be detected by RDT technology; these are: the Histidine Rich Protein II (HRPII) – found only in *P. falciparum,* Parasite Lactate Dehydrogenase (PLDH) and Aldolase.(12) Thus the HRPII antigen detection test is specific for *P. falciparum*, whilst the PLDH and the Aldolase antigen detection test can be used for all *Plasmodium* species (*P. vivax; P. ovale and P. malariae*). The antibodies in the test kits for antigen detection can therefore be species- specific.

MRDTs are manufactured in 3 formats.(22) The basic form is a dipstick, in which the absorbent nitrocellulose strip is placed in wells containing blood and or buffer. The nitrocellulose strip can either be placed in a plastic cassette or on a card. The mode of action for an MRDT is summarized in Figure 5.

1. Dye-labeled antibody, specific for target antigen, is present on the lower end of nitrocellulose strip or in a plastic well provided with the strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line, and either antibody specific for the labeled antibody, or antigen, is bound at the control line.



2. Blood and buffer, which have been placed on strip or in the well, are mixed with labeled antibody and are drawn up the strip across the lines of bound antibody.



3. If antigen is present, some labeled antibody will bind to the antibody on the test line. Excess-labeled antibody is trapped on the control line.

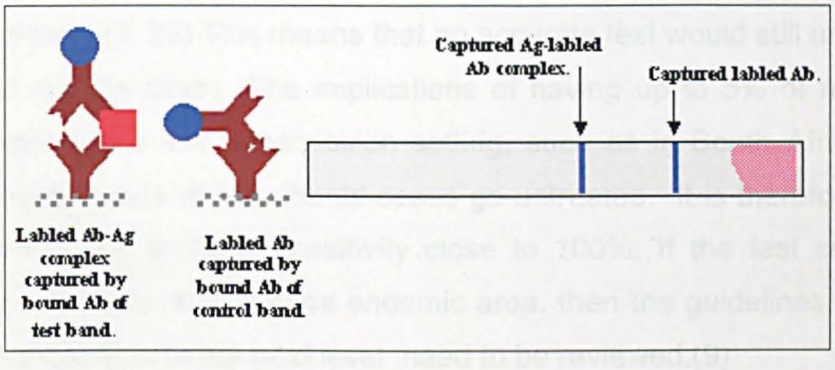


Fig 5: Mode of action of common malaria RDTs format, adapted from WHO Guidelines (23, 24)

Experts at a WHO informal consultative meeting on field trials and quality assurance of malaria rapid diagnostic tests, have listed several factors that may affect the accuracy of MRDTs, including: the manufacturing quality of MRDT; the environment (temperature and humidity) during transport and storage and the end-user's proficiency. (9)

2.3.2 Accuracy and field evaluations of MRDT's

Although HRPII antigen detection is useful for *P. falciparum* malaria diagnosis, the antigen persists in the blood of infected individuals for more than 4 weeks after successful treatment.(23) These tests may therefore not be useful for monitoring treatment success, as a false positive – which could be interpreted as treatment failure - is possible.(9) However if all MRDT positive cases are confirmed by microscopy as in South Africa the HRPII antigen detection test can be used as a screening test, for initial diagnosis of malaria .(7)

The accuracy of a MRDT is judged mainly by its level of sensitivity and specificity.(6) The World Health Organisation's guidelines on MRDT recommends that for an MRDT to be deemed 'accurate', it should have a sensitivity of ≥95% and a specificity of ≥90% at a level of ≥100 parasites per μl of blood using microscopy as the gold standard. (9, 23) This means that an accurate test would still underdiagnose up to 5% of malaria cases. The implications of having up to 5% of malaria cases under-diagnosed in a low transmission setting, such as in South Africa are grave and could result in loss of life, should cases go untreated. It is therefore imperative for a diagnostic test to have sensitivity close to 100%. If the test sensitivity and specificity is not 100% in a malaria endemic area, then the guidelines for treatment and referral of patients to the next level need to be reviewed.(9)

MRDTs that detect HRP II antigens are currently commercially available from several manufacturers and many have been evaluated globally in field and laboratory settings. In a systematic review of 32 studies of multiple settings,

Cruciani and colleagues reported an overall sensitivity of 90% and specificity of 94 % for Parasight F MRDT, an HRP (II) based antigen detection test to detect *P. falciparum* malaria.(25)

Results of studies on the diagnostic accuracy of ICT Pf MRDTs, the test currently used in South Africa, are summarized in Table 1 and 2. Iqbal and co-workers tested the accuracy of ICT Pf MRDTs in 515 patients in Kuwait using microscopy results as the gold standard and found a sensitivity of 82% and a specificity of 99%. (26) Singh and colleagues reported sensitivity of 100% and specificity of 85% in 353 patients in India.(27) Pieroni *et. al.* compared ICT Pf test and Parasight F test using PCR as the gold standard, among 200 febrile travelers in Toronto, Canada, and reported a sensitivity of 94% and specificity of 97%. (28) Thepsamarn *et. al.* found a sensitivity of 95% and a specificity of 92.7% in 305 participants in Thailand.(29) Among 98 participants Kumar and co-workers observed a sensitivity of 100% and a specificity of 100% using slide microscopy in another study from India.(30) Wonsrichanalai *et. al.* found a sensitivity 95% and specificity was 89% compared ICT Pf with malaria microscopy in 551 patients in Thailand using microscopy as the gold standard, (31) Bell et al. found in a study in the Philippines that the ICT Pf had a sensitivity of 98.7% when they tested 350 symptomatic patients however their specificity was low (74.1%). (32) Toma *et. al.* demonstrated in a study of 2 066 persons from a village in Lao PDR, that the sensitivity of the ICT Pf test was 92.2% and the specificity was 93.5%, see Table 1.(33)

Comparison of the sensitivity and specificity across settings is complex, due to the variability in epidemiological settings and reference standards; however Irwig et.al point out that there is indeed variability in the field accuracy of the ICT Pf test in different settings.(34)

2.3.3 Studies of accuracy of MRDTs in Africa

Studies in Africa also show variability in sensitivity and specificity of the HRP II antigen detection tests. Results of key African studies on HRP II antigens are summarized in Table 2.

Reference	Test	Country Setting	Sensitivity (%)			Specificity (%)	PPV (%)	NPV (%)
			Parasite density ≤100/µl of blood	Parasite density >100/µl of blood	Any parasite density			
(26)	ICT	Kuwait	NR	NR	82.0	99.0	98.0	83.0
(35)	ICT (Amrad ICT, Sydney)	India	NR	NR	100	98.7	95.0	100
(28)	ICT Pf Test	Canada	?	?	94.0	97.0	98.0	95.0
(29)	ICT	Thailand	?	?	92.7	95.0	93	96
(30)	ICT	India			100	100	95.4	100
(31)	ICT	Thailand	?	100	100	100		
(32)	ICT	Philippines			98.7	64	42	99.0
(33)	ICT (Amrad ICT, Sydney	Lao PDR	?	?	92.2	93.5	84.0	97

Footnote: ? - not included in the study; NR= not reported

Table 1: Accuracy of ICT Pf test for diagnosing *P falciparum* malaria outside Africa

Reference	Test	Country Setting	Sensitivity (%)			Specificity %	Positive Predictive Value %	Negative Predictive Value %
			Parasite density ≤100/µl of blood	Parasite density >100/µl of blood	Any parasite density			
(36)	Parasight™ F	Gambia 1	NR	NR	96.5	90.5	94.2%	94.3
(37)	ICT Malaria Pf	Uganda 1	71.4	100	100*	92.2	90.0	94.0
	Parasight F		71.4	97.1	97.1*	96.1	89.7	92.2
(38)	Paracheck dipstick	Uganda 2	86.3	98.9	97.4	88.1	91.5%	96.1
	Paracheck device		88.2	98.4	97.2	87.7		
	ParaHIT f		88.2	98.9	97.6	87.3	91.2	96.5
	Bio PF.		62.7	93.2	89.5	93.1		
	Malaria rapid		90.2	99.5	98.3	75.3		
(39)	Parasight™ F	Zimbabwe (Meso-endemic zone)	NR	NR	93.0	83.5	83%	87.5%
(40)	Parasight™ F	Tanzania	NR	NR	88.9	87.5	87.7	88.0

Footnote: NR= not reported in the study; * parasite density of ≥500 parasites /µl of blood

Table 2: Accuracy of HRP II antigen test in Africa

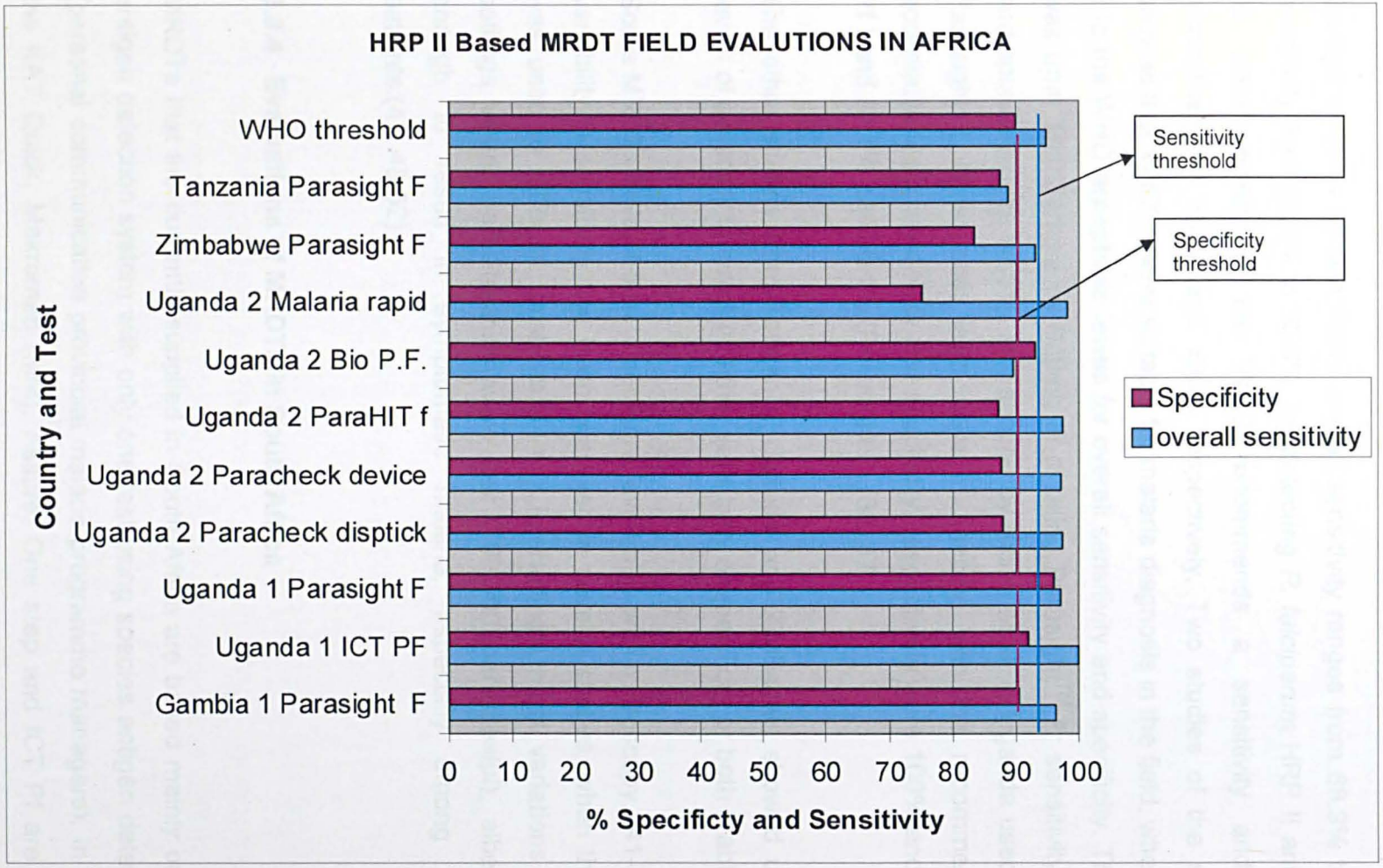


Fig 6: Specificities and Sensitivities of MRDTs in African settings against the WHO recommended thresholds.

Studies in Africa show MRDTs overall sensitivity ranges from 88.9% to 100% and specificity from 83.5% to 90.5%, for detecting *P. falciparum* HRP II antigen.(36-40) As stated previously the WHO recommends a sensitivity and specificity, respectively, of ≥95% and ≥90% respectively. Two studies of the six identified showed that MRDTs were suitable for malaria diagnosis in the field, when comparing it to the WHO acceptable levels for overall sensitivity and specificity. The first study was from The Gambia by Bojang *et al* using Parasight^{TM} F; sensitivity was 96.5% and specificity was 90.5%. The second by Killian et al. in Uganda used ICT Pf and Parasight F tests; both tests were in keeping with the recommendations for acceptable tests: sensitivity and specificity, respectively were 100% and 92.2% (ICT Pf) and 97.1% and 96.1% (Parasight F).(36, 37)

The other studies from Tanzania, Uganda and Zimbabwe showed unacceptable levels of accuracy in terms of either sensitivity or specificity or both (Table 2).(38, 39)

Some MRDTs have shown to compare favourably with microscopy. (41-43) However variability in sensitivity has been observed in several studies, when the same test was used in different locations.(44-47) Interestingly most variations occurred in settings where parasitaemias were low (100-500 parasites/µl), albeit significant enough to result in symptomatic malaria, especially among non-immune patients.(42, 48-52)

2.3.4 Evaluations of MRDTs in South Africa

MRDTs that are currently supplied in South Africa are based mainly on the HRP II antigen detection system with only one test using species antigen detection system (personal communication provincial malaria programme managers). In South Africa the KAT Quick, Makromed (MM), Assure, One step and ICT Pf are supplied for public health use. Kits are purchased on the basis of a tender system.

MRDT tenders should be awarded, using the main criteria of costs, test accuracy in the field and laboratory, stability of the test and ease of use of the test.(9, 53) The current MRDT in use in South Africa (ICT Pf) that was accepted in the tender did not undergo field evaluation for sensitivity and specificity on symptomatic untreated malaria patients. This was reportedly due to the fact that the tender needed to be awarded outside the malaria transmission periods, hence due to insufficient malaria patient numbers a field assessment was not possible.(7) The laboratory investigations of ease of use, diagnostic thresholds and multi-center health stability tests were however considered.(54) The heat stability results for the current MRDT revealed that the test could pick up parasites at levels of 100 parasites/ µl after 90 days exposure of the test at a temperature of 35^0C.

Durrheim et al. conducted a study in the Mpumalanga Province of South Africa to evaluate the accuracy and the usefulness of the ICT Malaria Pf^{TM} card test (ICT Diagnostics Sydney Australia) – a test previously used by the National Department of Health, South Africa.(55) The test was evaluated on 264 consecutive patients with signs and symptoms of malaria and the results were compared to microscopy. Their results revealed that the test sensitivity was 98.6% and the test specificity was 97.9%. Although the overall test accuracy was above the WHO acceptable threshold levels, the paper does not state the parasite detection level in the study setting.(9) This test is however no longer in use in South Africa as it was not selected by the National Department of Heath in its recent tenders.

Craig et al. evaluated the MRDT currently used in SA - ICT Pf MRDT; Global Diagnostics manufactured in Johannesburg, South Africa - to determine parasite detection limit in the laboratory using 19 serial dilutions of EDTA *P.falciparum* infected blood - ranging from 500 to 0.1 parasites/µl of blood). They also tested post-treatment specificity of the test. (56) The 50% detection limit of the ICT pf test was 3.28 parasites per µl of blood, whilst the test specificity post-treatment (following 7 days of treatment) was 30% In this study, however the ICT Pf kit was not evaluated for accuracy in a field setting.

Furthermore, the concentration of the target antigen in the host blood is one of the critical factors that affect the accuracy of MRDTs.(57) For a good test 100 parasites per µl is the acceptable threshold, hence in a low to moderate transmission setting such as in South Africa this may prove to be a challenge (58) as symptomatic patients with fewer than 100 parasites per µl are often seen in symptomatic non-immune patients in South Africa (58) and may be missed and thus remain untreated. It is therefore of vital importance to determine what percentage of patients with symptoms of malaria and a negative MRDT test are not treated and develop overt malaria.

2.4 End-user preparation and interpretation of results

End-user performance (preparation and interpretation) of MRDTs is key to ensuring accuracy of the test kits.(9, 59) To assess the end-user performance, WHO recommends that the ability of the end-user to prepare/ conduct the test and interpret the results should be determined. (9) A few studies were found in the literature evaluating self-administered MRDTs, especially among travelers.

Funk et al. assessed the Malaquick™ and the Parasight F MRDT in volunteers in Switzerland, with the key objective of determining whether lay persons could perform these tests and interpret the results correctly.(60) Volunteers were asked to perform the test with their own blood using the test instructions. There was no significant difference between the two tests in end-user ability to perform them: 87% of participants performed the Malaquick correctly and 71% performed the Parasight F test properly. Participants identified blood collection – especially finger pricking - and identification of test components as practical difficulties experienced during test performance. The overall test scores for interpretation did not differ significantly between the tests when the prepared test strips had a parasitaemia of 0.1% and 2% but major problems were encountered at low level parasitaemias (<0.1% blood parasites). A false negative interpretation of 72% (Malaquick) and 29.6% (Parasight F) was a major concern in this study as these cases could go untreated. Approximately 40.1 % of the study volunteers were considered to have a high level

of education, being at university. In spite of having the instruction manuals for the tests, 20-30% of participants did not perform the MRDTs optimally and interpretation of tests was poor, especially for the low-level parasitaemia's. Clearer instruction aids and examples of prepared test results may be one way of addressing the challenges highlighted in this study.

Whitty et al. conducted a study in the UK among sick travelers seeking medical attention at a travel clinic to determine their ability to perform the ICT Malaria Pf using only the instruction manual and no oral instructions.(61) They were mainly interested in whether febrile patients with symptoms of malaria could complete the test (ICT Malaria Pf) satisfactorily, and interpret the results correctly under simulated field conditions (assuming no medical assistance will be available). A questionnaire was also administered to determine whether travelers familiar with the tests would use them in the field. Eleven percent (6/153) of symptomatic patients presenting with malaria failed to correctly perform a test (4% patients were unable to use the lancet, 3% obtained insufficient blood, 4% could not read the card). From those patients who satisfactorily completed the tests the results of their self-diagnosis was compared to that of slide microscopy. The specificity and sensitivity of the self-testing was 97% (95% CI-93-99%) and 95% (95 CI-74-99%) respectively. Of the 153 people enrolled in this study the first 107 commented on the instruction manual, and their suggested changes were incorporated into the manual. After changing the manual, the percentage of people who indicated that the test was difficult to interpret decreased from 20% to 7% (p=0.004), in the 46 patients subsequently tested.

Trachsler et al. conducted a study in Switzerland to determine whether untrained travelers could successfully perform and interpret the results of MRDTs (using the Parasight F test).(62) The study involved 160 consecutively selected visitors attending a travel clinic in Zurich, whereby one group (n=80) received written instructions and the other (n=80) written and verbal instructions on how to test their own blood for malaria and interpret the results. The group that received both oral and written instructions performed the test significantly better than the group who

only received the written instructions 72/80 (90%) achieved satisfactory results, versus 60/80 (75%), p<0.0). The key issues for poor performance as cited by the participants were the incorrect positioning of the dipstick and difficulty with taking blood; the latter was experienced by 107 (66%) participants. In the next stage of this study each of the 160 participants were also given 5 test strips to interpret and they correctly interpreted 566 out of a total of 800 options (70.6%). There were no false positives but there were 113 false negatives (9 for dipstick positive, 53 for dipstick weak positive, 40 for dipstick massive positive and 12 for dipstick no result). The key challenge identified by the authors was the unavailability of clear instructions with graphic illustrations of the test results. False negative results in this study is again cause for concern; presuming these travelers were to act on the results, then there is a chance that 102 (dipstick no result would probably be retested) persons with malaria could potentially have gone untreated.

Tavrow et al. in the first of a 2-phased study in Malawi, assessed how 19 health care providers (end-users) performed MRDT tests (Path and Flow): 8 of the end-users received training prior to receiving the tests and the other 11 did not. (63) Their findings showed that overall 15% (3/19) performed the test without any errors; those that were trained performed better (25%- 2 of11 in trained vs 9%1 of 11; in untrained). In the second phase of the study a new group of 20 participants (using similar characteristics of participants as the first group) were selected, no training was provided but redesigned instructional inserts were used based on the gaps identified from phase 1 of the study. Eighty-five percent off end-users (17/20) performed all the steps in the test procedures – a significant improvement from the previous group. End-user interpretation of results was at 85% in phase 1 of the study and reportedly improved remarkably during phase 2 (no data was presented in the paper though). Although this study was conducted in a small number of participants and therefore findings cannot be generalized, it does give an important indication of the key interventions that could improve end-user performance and interpretation of MRDT results.

A study by Maxay *et al.* showed that 64 village health volunteers performed 2 malaria MRDTs (Optimal and Paracheck Pf test) with a high level of accuracy in the Lao People's Democratic Republic.(64) The village health volunteers were given one hour's training on the performance and interpretation of the MRDTs. They were assessed on the same day of the training for their ability to perform the test and interpret the results on 3 blood samples, one positive for *P. falciparum*, one positive for *P.vivax* and one negative for all parasites. No written or pictorial materials were provided and participants did not take notes. The mean test performance scores were 99.6% for Optimal and 99.0% for Paracheck™ *Pf*. Training as an intervention (without instructions or pictorials for lay persons) may have given them skills on how to accurately perform and subsequently interpret the results. The element of bias could have crept in as these were enthusiastic motivated village health volunteers that participated in the study. The skill retention beyond 10 months was not assessed in study, hence it cannot be established whether performance is sustained or whether retraining and the use of instruction materials would have improved performance.

Rennie *et al.* conducted a study in the Philippines (n=152) and Laos (n=107) among community health workers, to determine the effect of job aids on end-user performance using malaria rapid diagnostic tests. The study was conducted in 2 phases: the first group of participants used the manufacturer's instructions (English and local language) to conduct the MRDTs: their performance was judged using a standardized checklist. Subsequently, using the knowledge of the gaps identified from the initial observations a detailed pictorial job aid with clear instructions was developed. Observers used the same checklist to test the pictorial job aid in the Philippines on a different group of participants. This job aid then underwent minor modifications and was used for testing in Laos. In Laos, two groups of participants were randomised to either using the job aid alone or to the job aid plus one hour training. In this study, although the mean overall performance of end-users in Philippines and Laos improved significantly after introducing the pictorial job aid (compared with the initial performance of the Philippines group) performance still

remained below 80% in both study sites. In the Philippines the mean percentage of correct steps using the manufactures instructions was 42% and this improved to 59% when the job-aid was used and in Laos it also improved from 58% to 68%. The group in Laos that had prior orientation coupled with use of job aids versus the group with the job aid only, performed the test better (mean of 80% vs 70%; $p < 0.001$). The key challenges to performance and interpretation of the results listed by the authors in the study include checking expiring dates, kit desiccant and interpreting the weak positive and negative results. This study highlights the need for all 3 key interventions (baseline assessments of end-user proficiency; development and testing of job aids and training coupled with job aids) to be considered for MRDT implementation.

In South Africa no formal studies have been conducted to assess end-user proficiency to perform and interpret MRDTs. The exploratory study conducted in March 2006 as formative work for this thesis (see Chapter 3 for details) revealed huge uncertainty among health managers in the Limpopo Province about end-user MRDT proficiency, especially at the primary health care level. (7, 65) A study to determine the end user proficiency would therefore prove invaluable to determining the current levels of accuracy among end-users. This would also provide useful information to decision makers on key areas that need to be addressed to improve the performance of MRDTs at the PHC level, should there be a need.

2.5 Quality control of MRDTs

As discussed in sections 2.2.6 and 2.3.2 and under or over-diagnosis of malaria could have grave consequences. There is therefore a need to ensure that MRDT quality control is maintained according to the WHO acceptable criteria.(9) The WHO defines quality control as "all the activities taken by a laboratory to monitor each stage of a test procedure to ensure that tests are performed correctly, accurately and precisely." (66)

As explained in section 2.3 and Figure 5 MRDTs are lateral flow immuno-chromatographic devices, which contain antibodies to capture specific antigens (HRPII or LDH). (6, 9)

By virtue of this MRDTs can be degraded by heat or moisture and can deteriorate even in ideal conditions. A positive control line on the test kit confirms migration of the dye-antibody conjugate on the test strip. It does not confirm the ability of the antibodies to bind to malaria parasite specific antigens, nor adherence of the test-line antibodies to the nitrocellulose, nor does it signal moderate de-conjugation of the anti-parasite dye antibody conjugate. An MRDT may still therefore show a positive control line despite inadequate sensitivity affected possibly by temperature and humidity. Temperature or vaccine vial monitors may be used to indirectly monitor temperatures of the test kit condition. Furthermore variation in MRDT accuracy in published trials and operational experience highlights the need for a system to monitor the accuracy of the tests after they have been dispatched from the manufacturer.(66) It is therefore of paramount importance to have some quality control system in place to ensure that the test kit is working post- field exposure.

The WHO recommends the use of prepared quality control samples using wild type parasites for testing of malaria rapid diagnostic tests, especially at the laboratory level.(66) However the sophistication of the methods involved in preparing and storing wild type antigens puts this technology beyond the reach of district laboratories and remote health facilities - especially in developing countries where the malaria is most prevalent , hence justifiably this technique is not recommended by WHO for use in quality control. Ensuring MRDT quality at the post-manufacturing stages can be established in a central sophisticated laboratory with the necessary skills and the equipment, laboratory; however at the remote health facilities level alternative quality control interventions are required.(66)

There are a few possibilities available for remote quality control (assuring quality in the field only) testing of MRDTs as outlined by the WHO (66); these include:

- Sentinel site quality control: Comparison of results in a small number of "sentinel" sites with microscopy, using slides stained on site and read centrally and MRDTs which have undergone typical storage and distribution, to ensure that they remain adequately sensitive;
- Using record books: Health workers can record: symptoms, MRDT results, their interpretation of findings, treatment and outcome post follow-up – this can then be reviewed by a supervisor and
- Positive Control Wells (PCWs), using lyophilized recombinant antigen, at first referral level. This could be used to periodically evaluate batch test performance over its shelf-life under local field and storage conditions.

Sentinel site quality control assessments for remote health facilities, whilst possible in South Africa, may not be beneficial as faulty batches of MRDTs will not be detected instantaneously. Similarly the use of record books may result in delayed action to remove faulty batches of MRDTs from health facilities; moreover it would require additional human resources to undertake the supervisory roles.

The use of PCWs, containing lyophilized recombinant antigens for assessing MRDT quality in the field is an attractive option for South Africa to consider.(67) The procedure once established will not require sophisticated technology and skills, although it will require that senior nursing staff at health facilities be trained on how to appropriately use the wells.

PCWs are currently not being used in operational settings, as the stability of the wells are still being assessed.(67) Published studies on assessing the field stability and operational practicalities of PCWs are rare. One study was found in the literature, by Lon et. al. who used a commercially available pLDH PCW to assess it's ability to monitor MRDT sensitivity in a remote malaria endemic area of

Cambodia.(68) In brief antigen strength of the pLDH wells stored at 4^0C at a centralised laboratory were compared with those un-used wells kept in the field. Their results showed that there was no significant difference in the intensity of the lines between the reconstituted wells stored centrally at 4^0C and those stored in the field for 8 months. These results indicate that there is potential to use antigen-coated wells to monitor MRDT sensitivity in the field. However this pLDH positive control well testing system will not be appropriate for South Africa as the South African MRDTs are HRP II- based, not LDH-based.

Since 2006 a commercial company (National Bio-products Institute - South Africa) is manufacturing the HRPII antigen in the laboratory and has undertaken laboratory testing for temperature stability and to determine the appropriate diluent for reconstituting the antigen (citrated HRPII-negative blood or buffer). This therefore opens the way for the field testing of the HRP II antigen positive controls as a potential quality control tool for monitoring MRDT sensitivity at field sites in the Limpopo.

The variability in MRDT sensitivity and specificity of HRP II found in African and non-African studies and the implications of the consequences for misdiagnosis, justifies the need for a MRDT evaluation in South Africa.

2.6 Aims of the study

This study aimed to address 4 key research questions:

(1) What are the key factors that affect the quality and use of MRDTs in the Limpopo Province?

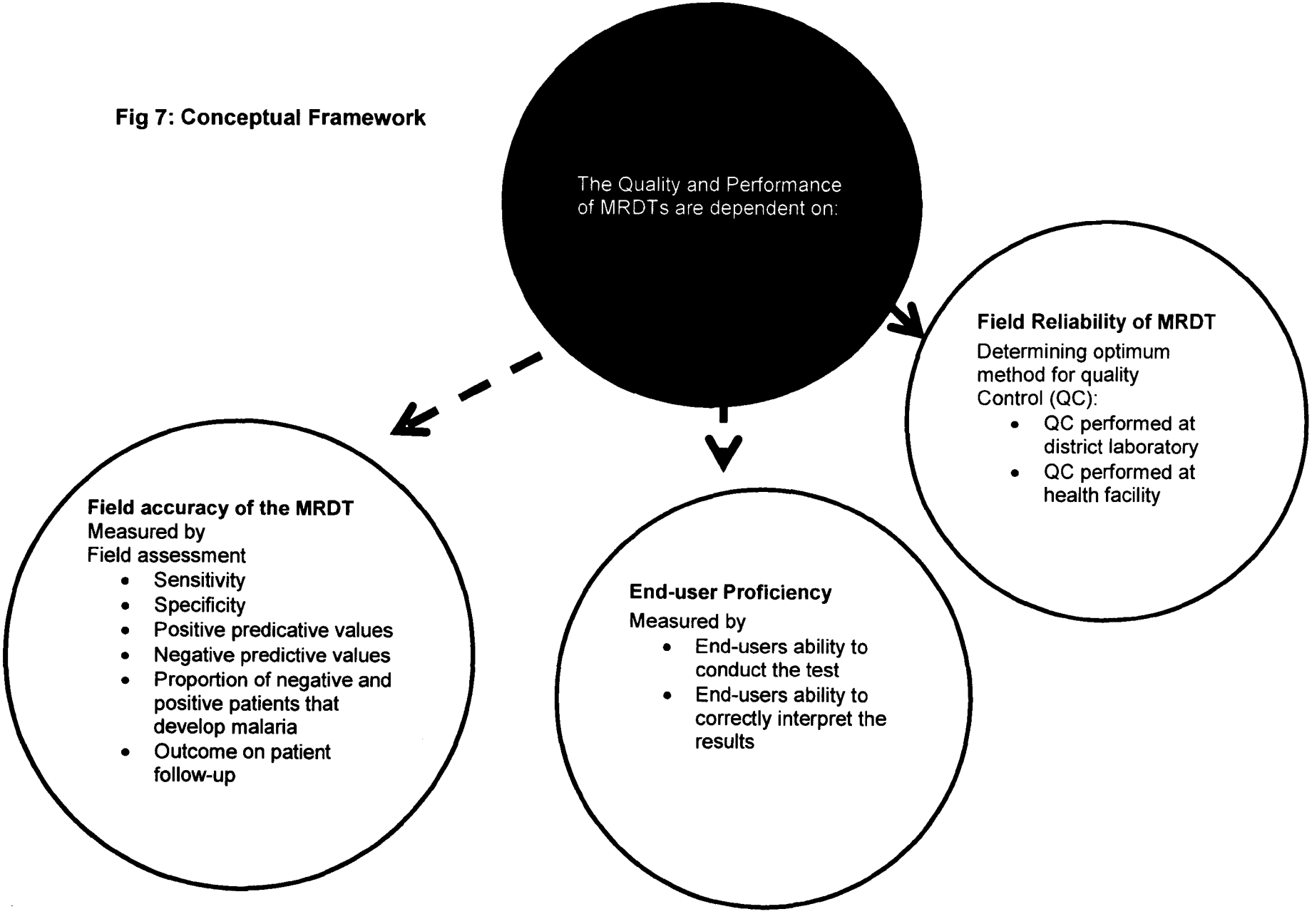
(2) What is the field accuracy of the current MRDT test to diagnose malaria in the Limpopo Province? What proportion of patients with a negative MRDT develop malaria?

(3) What is the end-user's ability to accurately perform and interpret the MRDT results in Limpopo?

(4) Whether PCWs could be considered for a quality control programme for routine sensitivity monitoring of HRP II antigen-detecting MRDTs in the Limpopo Province

See Figure 7 for the conceptual framework for the study.

Fig 7: Conceptual Framework



After reviewing relevant literature (9) the two key issues that were deemed to be critical for correct MRDT performance and usage were MRDT quality and end-user proficiency; thus these were the focus of this study. Logistical and operational weaknesses identified in the exploratory study, such as stock-outs and temperature monitoring, though important, were communicated to the relevant policy makers at the Limpopo Provincial Department of Health, for remedying, and were thus not the main focus of this DrPH.

3.0 Chapter 3: Factors that affect the quality and usage of malaria rapid diagnostic tests in the Limpopo Province, South Africa

3.1 Introduction

This study was undertaken as formative work for this thesis. It aimed to identify the factors that, in the opinion of end-users (nursing staff) and other health care providers (key informants), affect the usage and performance of malaria rapid diagnostic test kits (MRDTs) at primary health care level.

3.2 Methods

This was a cross-sectional study,semi-structured interviews were conducted to obtain the perspectives of end-users and key informants. Responses were documented through notes taken during interviews.(69) Perspectives on purchasing, storage, transport, usage (including training, ease of use and confidence with results) and quality control in Limpopo were obtained.

3.2.1 Sample

The sample comprised a total of 20 end-users and 10 key informants. End-users comprised 17 clinic and three hospital nursing staff. The key informants included three hospital pharmacists, one regional pharmacy manager, one provincial malaria control programme manager, three district malaria managers and two researchers. The three district managers and the three hospital pharmacists were purposely selected from districts that had the highest number of malaria cases (Bohlabela, Mopani and Vhembe for the years 2002-2005). One district malaria manager was interviewed from each high risk district that had the highest incidence of malaria. The two researchers selected were purposefully selected from the national research laboratory- providing quality control support on MRDTs to the provincial malaria control programmes of South Africa.

A two stage sampling procedure was used to select PHC clinic staff.(70) All clinics and health centers within the three malaria-affected districts were listed. Clinics and health centers with fewer than 10 malaria cases per annum were excluded from the sampling frame. In the first stage of sampling, 10% of the clinics (10/100) and 20% of health centers (7/35) within each selected district were randomly selected (see Appendix 1). In the second stage of sampling one nursing sister was randomly selected from each selected health facility. Five referral hospitals were purposefully selected, one from each of the major municipalities that housed the selected clinics.

3.2.2 Data collection and analysis

The end-users and key informants were interviewed using a semi-structured interview schedule, with a separate interview schedule for each group (see Appendix 2). The interview schedule was developed after reviewing key WHO literature (9, 66) and other relevant published literature on MRDT end-user proficiency.(62, 63) Consultation with international experts, laboratory and malaria managers from the provincial and national levels also guided the development of the interview schedule.

The end-user interview schedule was piloted on three nurses from non participating health facilities, prior to using them for data collection. Similarly the key informant interview schedule was piloted with two district malaria managers that were not selected for the study.

The student conducted and transcribed all interviews and then ordered and coded the results in matricies, using the key categories of procurement & stock monitoring, storage, transport, quality control and end-user experiences with using MRDTs (including training, ease of use and confidence in results).(71) Nurses were asked to rate (as a percentage) their level of confidence with the MRDT results. Their responses were divided into three groups; low confidence was less than 50%; medium confidence was 50-80% and high level of confidence was greater than 80%.

3.3 Bias

Information bias may have occurred during the interviews as participants may not have trusted the student (interviewer), as participants knew that the interviewer worked at the Ministry of Health, and some may have felt that the interviewer (student) required information for the media or for the National Department of Health.(72) The interviewer attempted to overcome the information bias by adequately introducing the purpose of the study, allowing the respondents to ask questions and assuring the respondents of the confidentiality of their information.(69) All the interviews were conducted by the student (DM) and therefore there is no inter-observer bias. Intra-observer bias was minimized by asking all questions in exactly the same order and by using the same phrases and questions for each interview (see Appendix 2 for interview schedules).(73)

Ethics

No personal identifiers were attached to any of the materials used for semi-structured interviews. Confidentiality was maintained at all times, whereby all data materials were locked in steel cabinets and the student had access to this information only. The University of Limpopo Research Ethics Committee (project number 32/2006) and the Limpopo Department of Health and Welfare (ref 4/2/2) and the London School of Hygiene and Tropical Medicine (reference 3058) granted approval for this component of the study. Informed consent was acquired from all participants using participant information sheets and consent forms (see Appendix 3).

3.4 Results

Nurses informed the student that in a routine set-up, MRDTs are used as a routine diagnostic tool for malaria, and all treatment was based on MRDT result. As per the malaria diagnosis protocol in the Limpopo province a thin and thick blood film were subsequently made on every MRDT positive patient for malaria and sent to the malaria control progamme for confirmation of diagnosis.

3.4.1 Procurement and stock monitoring

Nursing staff reported that they were monitoring and ordering their stocks and checking the expiry dates regularly. The process predominately involved ordering either from the hospitals, in the case of Vhembe and Mopani districts or ordering from the amalgamate pharmacy stores at the provincial depot in Polokwane, for the Botshabelo district. Only 20% of the health facility nursing staff were aware of the seasonal increase in malaria cases and these stated that they ordered more MRDT stock before and during the season (September to May). Stock monitoring ranged from stock cards (paper based) to electronic systems. Pharmacy staff reported that pharmacy assistants were sent to some clinics and health centres to assist with ordering of pharmaceuticals, including MRDTs. Some hospitals e.g. Phalaborwa Hospital in Bohlabela district had a barcode system for ordering pharmaceutical supplies and it was working very efficiently.

In spite of all the systems in place, 55% of the nursing staff indicated that their health facilities ran out of stock during the peak malaria transmission periods. Most of these nurses stated that during stock-outs there were plans in place to get additional stock in an emergency, from the next clinic or from the hospital, Malaria managers confirmed the clinic-level stock-outs in the 2005/2006 season (July to June). Managers indicated that they alerted the necessary authorities (district managers) to take action. Malaria managers transported the malaria kits to the clinics and health centers during the epidemic periods. However they stated unequivocally that it was the Primary Health Care Manager's responsibility, and not their responsibility to transport MRDTs; however they were willing to assist where possible.

The regional pharmaceutical manager was not concerned about stock outs at the regional pharmacy level indicating that an electronic system was in place to increase supply as the demand for stock increased, and in most cases this was proportional to the seasonal increase in malaria cases.

3.4.2 Storage of MRDTs

Sixty-five percent (13/20) of the end-users said that they stored MRDTs in an air-conditioned room and regularly monitored temperatures, and seven (35%) had no access to an air-conditioned room. Of these seven, three (42%) did not have a thermometer for monitoring room air temperature and were very concerned about air temperature fluctuations in the storage room; whilst 4 of 7 reported that they monitored storage room temperature regularly and that this rarely went above 30°C. This was however not corroborated with any recorded data.

The malaria management staff from the Vhembe district confirmed that some clinics did not keep the MRDTs in a cool environment; however they were not too concerned about this, stating that: "*the kits did not stay in the clinics for too long due to their frequent use.*"

The hospital pharmacists and regional pharmacy manager stored the MRDTs in an air-conditioned environment, at a room air-temperature ranging between 15-25°C. Monitoring charts were produced on request.

3.4.3 Quality Control (accuracy of MRDT results)

The majority of the nursing staff- 80%(16/20) - reported that they did not check the quality of MRDTs by comparing with any perceived Gold Standard, commenting that they "believed" the MRDT results. Four of the 20 nurses reportedly checked quality by looking at the agreement between their diagnosis based on signs and symptoms and the MRDT results. Two of these four indicated that they occasionally compared the MRDT results with those of blood smear results.

Fifteen percent (3/20) of the nurses indicated that they occasionally gave antimalarial treatment to MRDT-negative patients on the basis of clinical signs and symptoms of malaria. Box 1 highlights the key challenges identified by nurses,

researchers and malaria managers relating to MRDT quality control including correlation with smears and clinical signs and symptoms.

In summary, all managers were very concerned that MRDT quality was not being monitored at health facility level. One informant stated that, "*MRDT quality control, both at the manufacturing side and at the testing stage, was lacking. The key [issue] is the end-user's ability to distinguish between positive and negative results*".

Quality control from nurses' perspectives : selected quotes

- *"our results did not correlate with the patients' signs and symptoms"*
- *"our results did not correlate with the lab findings"*
- *"unsure of a negative results"*
- *"we find it positive they (lab) find it negative"*
- *"clinically suspected cases were negative on RDT but positive in the hospital"*

Quality control from the perspective of researchers and malaria control programme managers

- *"...accuracy of the test, we are not getting the required level of sensitivity;"*
- *"No commercial control is available;"*
- *"we are concerned about the stability of the quality control specimen;"*
- *" the key challenges are operator level efficiency, quality of the actual test, training instruction in the tests itself and performance of the test in the field"*
- *"finding the right specimens and sources of the specimens for quality control, poses a huge challenge"*
- *"it is difficult to try to tease out what the problem really is in doing the test, ensure that the variables are all the same when testing proficiency."*
- "sometimes *smears are done in parallel with the MRDT and sometimes there is discrepancy.*"

Box 1: Quality-related challenges: selected quotes by nurses, researchers and malaria control managers

3.4.4 End-user experiences with using MRDTs

Forty percent (8 of 20) of the end-users (health workers) reported that they had received training in using MRDTs. However only two of eight trained users had received training from the malaria control programme, the remaining 6 users received regular in-house training from colleagues.

Sixty percent (12/20) of the users said that they did not receive any outside training on MRDTs for malaria: however when probed, ten of the users (83%) said that they were aware of in-house training in MRDTs.

The district and malaria managers reported that training did take place when MRDTs were first introduced; however subsequently limited training took place. This limited training was in high-risk clinics in response to requests from health facilities.

Sixty five percent (13 of 20) of end-users said that they did not follow any provincial or district guidelines for using MRDTs. Most end-users including those who reported not using a provincial or district guideline reported using the package insert as a guideline for MRDT-use.

One hundred percent of end-users indicated that they found the test very easy to use. When asked to rate the ease of use of the MRDT, 90% of nurses gave the ease of use five marks out of a total of five, whilst 10% gave the ease of use 4 marks out of a total of five. The 10% did not have any serious concerns but were reluctant to give full ratings. Thus in the view of the end-users (health workers) MRDTs were considered easy to use.

End-users were confident with their ability to interpret results. Some of the concerns raised albeit rare, were that there was blurring of the test lines, or in one instance no lines appeared. The end-users reported that when a MRDT result was unclear they repeated the test and the results of the repeat test became clearer to interpret.

End-users were also asked to what extent they believed the MRDT results they obtained and responded as follows:

Almost all (95%) of them said that they were confident with the MRDT results they obtained.

Seventy percent of them (14) were more than 80% highly confident and the remaining 20% were 50-80% confident (the medium category confidence) with the results they obtained. There were no responses in the low category confidence levels.

See Box 2 for some of the key concerns among respondents.

- *"Our rapid test results do not correlate with the patients signs and symptoms"*
- *"Our rapid test results did not correlate with the lab findings"*
- *"We are confident with positive results but in 15% of the time we are unsure of negative results"*
- One respondent in the medium category (50%-80% confident) indicated that *"seventy percent of the time our results matched that of the hospital for positive cases and 30% of the time we find it positive they finding it negative"*
- One respondent from the medium category indicated that *"I am 70% confident, as sometimes the clinically suspected cases were negative on MRDT but positive in the hospital"*

Box 2: Key concerns from nursing staff regarding confidence with MRDT results

Although there was indeed a high level of reported confidence in the use of MRDTs and in their results, false positive and false negatives were key challenges experienced by the end-users. In some instances results were as much as 30% discrepant (laboratory findings did not agree with MRDT results).

All the participants fully agreed that the MRDT was easy to use, rapid and could be used in the primary health care setting with immediate benefit to the patients - see Box 3 for some of their key responses.

- *"It is rapid easy to use and it can enable treatment and can prevent complication."*
- *"Test is easy to use a nurse can make a diagnosis without involving the doctor and treat the patient".*
- *"The community is aware that such a test exists, they come to clinic and ask for a malaria test."*
- *"We can do the test at the clinic no need to go to the hospital."*

"I can praise it because it can save someone's life."

Box 3: Nursing staff responses to positive aspects of using MRDTs

The end-users reported the following negative aspects of the MRDTs

- Accuracy of result was the key concern for 35% (7/20) of the respondents, false positive and negatives were major challenges.
- Three participants were keen to get test kits that could detect malaria in less than 15 minutes.
- Three participants mentioned that mixed infections were a challenge.
- Two participants were concerned with the buffer being inadequate for the test.

Malaria managers commented that reading and interpreting MRDTs were often problematic, stating that "*the fault could have been due to the clinic staff not reading the test in time.*" One key informant (laboratory staff) felt that the end-users were not doing the test properly stating that "*When we receive the test back we see that they are putting too much blood.*"

3.5 Discussion

3.5.1 Procurement and stock monitoring

It was apparent that ordering and monitoring of MRDT supplies at the clinics were efficient, however the occasional MRDT stock-outs, is a cause for concern. Planning for having adequate stock especially over the peak transmission period is vital to preventing stockouts. Liaison with the hospital pharmacies and the depots is therefore crucial to ensure a steady stream of stock replenishment.

3.5.2 Storage

The manufactures recommendations for storage of ICT MRDTs was below 35^0C (ICT diagnostic package insert) as the heat stability tests showed consistent positive bands at 35^0C for storage periods for up to 0 and day 9 at parasite concentrations of 200, 500 and 1000 per µl of blood.(9) According to the WHO and other studies, temperatures above 30^0C are considered inappropriate for storing MRDTs.(66, 74, 75) In the study sample only 65% (n=20) of the clinics had air conditioners and 85% (n=20) were monitoring temperatures.

In the summer months during high rainfall and peak malaria transmission there is an increase in demand for MRDTs at the clinics and the average temperatures in the Vhembe district ranges from 38-42^0C (South African weather Services). Increases in temperatures above 30^oC can affect overall performance of MRDTs and the efficacy of drugs (including the anti-malarial drug Artemether-Lumefantrine) which is stored in the same room as MRDTs. It therefore becomes important that room temperatures are maintained below 30^0C and temperature monitoring is takes place regularly in the MRDT storage room of all clinics. Those rooms that exceed temperatures of 30^oC should be cooled with air-conditioning or similar cooling equipment, this being dependent on financial resources available to local health authorities in the study districts.(9)

3.5.3 Quality

There was huge uncertainty in the quality control and accuracy of MRDTs by end-users, malaria management staff and research laboratory staff. Furthermore, the fact that the nursing staff sometimes "believed the results", in spite of the test kit not having a positive control and their results being discrepant with the laboratory findings, is cause for concern.

Discrepancies between the laboratory and end-user findings that were a concern for 4/20 participants and 3 key informants could point to two challenges: either the test kits were not working or the end-user was not using the kits correctly.(9, 19, 59, 67)

The practice of treating patients, who had a negative malaria rapid test, as reported by 15% of the participants, raises the possibility of lower sensitivity of the existing MRDT (false negatives among MRDTs). This may be either as a result of faulty MRDT or incorrect use of the test by the end-users, these hypotheses require investigating.

Due to uncertainty around the quality of the test and lack of confidence in some instances of interpreting the results, respondents reported referring patients to the next level of the health care system as per the current malaria treatment and prophylaxis guidelines.(8, 76) This may cause overload at the next level and may be detrimental to the patients if they were not correctly treated prior to, or at referral. Detrimental effects could lead to rapid disease progression and even mortality, due to either long waiting times (peripheral hospitals in the Vhembe district is often understaffed and see large numbers of patients – personal communication with the Vhembe District Health Manager) or failure of the patient to go to the next referral level (hospital).(9, 59, 67)

3.5.4 End-user experiences

External training was not being conducted for the end-user and in-house training was not based on a standardised curriculum using standardised materials and methods. There is also uncertainty on the quality of existing in-house training for MRDTs. A standardised training guide for in-house training may need to be considered.(9, 59, 63)

Although package inserts are useful it would be easier for end-users to have posters or job aids so that the test procedure and can be easily visible and interpretation of results easily read especially during busy periods and late in the night. (9, 59, 67)

The uncertainly about the skills of end-users to use MRDTs from both the malaria managers and the laboratory staff is indeed a cause for concern. Proficiency testing should be considered, to scientifically evaluate the skills of end-users.

3.6 Conclusions

It is clear that the value of the MRDT for diagnosis of malaria at the primary health care level was understood by all the categories of respondents: end-users, malaria managers, pharmacy managers and laboratory staff.

In this exploratory study the procurement management and distribution of MRDT tests was found to be efficient. The storage of malaria rapid test kits was not entirely satisfactory: temperature monitoring will need to be conducted to bring this component to more acceptable standards.

The key challenges for malaria MRDT in the Limpopo are the accuracy of the kit, the proficiency of end-user and quality control to ensure that the kits are working post-field exposure.

See Appendix 22 for scientific publication of this chapter.

4.0 Chapter 4: Accuracy of malaria rapid diagnostic tests in South Africa

4.1Introduction

The current malaria treatment policy in South Africa, stipulates that treatment for malaria, should be administered to patients, subsequent to definitive diagnosis. (8) High levels of sensitivity (>95%) and specificity (>90%) of MRDTs are key to ensuring accurate malaria diagnosis. The reported sensitivity and specificity of MRDTs, from other settings varied substantially.(29, 31, 37-40, 45, 47, 77, 78) Thus a field evaluation to determine the accuracy of the MRDT currently used in South Africa is needed to inform policy on the diagnosis of malaria. Such an evaluation will set precedence for, and guide future tender evaluations of MRDTs by the National Ministry of Health - a process that occurs every 2 years and has until now occurred without field evaluations of the MRDTs on tender.

This chapter provides a detailed report on assessing the accuracy of the MRDT currently used in South Africa (ICT Pf- Global Diagnostics) and on the treatment outcomes of cohorts of MRDT-positive and negative patients.

4.2 Study Objectives

4.2.1 The primary study objective was to:

- determine the field accuracy of MRDTs (ICT Pf Global diagnostics) used in South Africa.

4.2.2 The secondary objectives were to:

- determine the accuracy of MRDTs in a low density malaria setting;
- describe the clinical outcomes of Malaria RDT- positive and negative patients and
- determine clinical predictors of malaria in this study population.

4.3 Study Methods

4.3.1 Study design and sites

This study had two components. The first component was a cross-sectional study of patients, presenting to selected health facilities, with suspected malaria to determine MRDT accuracy. The second component was a prospective observational cohort study to determine the clinical outcomes among MRDT-positive and MRDT-negative patients (sick with malaria, hospitalized or dead). MRDT positive and MRDT negative patients were followed up at the health facility or located at their homes on day seven post MRDT testing.

The Vhembe district was chosen because it had the highest incidence of malaria for the past nine years (see Figure 3) prior to the start of this study.(10) Two clinics from the Vhembe district: Madimbo and Mulala were selected (see Appendix 4), based on the highest and second highest mean number of malaria cases over the 3 years prior to the commencement of this study, this was determined using national malaria notification data.

4.3.2 Study population including, inclusion and exclusion criteria

For the cross-sectional component of this study, the study population comprised consecutively-selected, male and female cases of suspected malaria (fever or headache or chills) - in keeping with the national malaria treatment guidelines for South Africa - attending the study clinics for an initial visit and who consented to participating in the study. Patients attending the study clinics for a follow up visit, severely ill patients needing referral, patients with an obvious cause of fever, and pregnant women were excluded from the study.

For the second component of this study, all MRDT positive patients and 10% of the MRDT negative patients detected during the cross-sectional component described above were followed up on day seven post malaria rapid testing and treatment, to determine their clinical outcomes.

4.3.3 Sample Size

It was assumed that the sensitivity of the MRDT should be 95%, the level recommended by WHO to be useful. To estimate 95% sensitivity with 95% confidence limit of +/- 3%; 203 cases of suspected malaria per clinic. – giving a total sample size of approximately 405 for two clinics - see Appendix 4.(79)

4.3.4 Data Collection

The author (DM) developed standard operating procedures (SOPs) and data collection tools to ensure collection of good quality data (see Appendicies 5-8).

Each patient was given a unique identification number (ID) and this was used to label the data collection forms, the MRDTs and the thin and thick blood smears (see Appendix 5). At enrolment demographic information, clinical symptoms and signs and the results of the MRDT were recorded on standard recording forms: one form for the initial visit and a second form for the patient follow-up visit (Appendix 8). Nurses performed an MRDT on each patient who met the inclusion criteria after obtaining a written informed consent (Appendix 9). Nurses also made thin and thick blood films after performing an MRDT. Blood films were sent to two specialized trained malaria microscopists at the Limpopo Department of Health (Thulamela health center at the Vhembe District) for staining and microscopy.(34, 80)
Patients were given recall cards that were also developed in the local language (Tshivenda) to remind them about the date on which they needed to return to the clinic for a follow-up visit (see Appendix 7).

Blood films were stained with giemsa using the standard WHO protocol Two hundred thick film oil immersion high-power fields were examined before a slide was interpreted as microscopy negative for malaria. Parasite densities were calculated by counting the number of asexual parasites and multiplying this figure by the

standard white blood cells count (8000/ µl of blood) and the resulting value was then divided by the total white blood cells counted during the microscopic examination.
Two field microscopists who were blinded to the MRDT results read the thin and thick blood films independently. When there was discordance between microscopists and MRDT results, a third highly skilled microscopist (based at a reference centre) blinded to the MRDT and the field microscopists readings, read the discordant slides. Discordance was settled by the microscopy results obtained by the highly skilled microscopist from the reference laboratory.(34, 70) To determine the agreement of slide results between microscopists (field and reference centre), Cohen's Kappa statistic was used to determine microscopy reader reliability, a score of ≥ 0.8 was considered reliable.(9, 81)

4.3.5 Data management and analysis

The primary outcomes to assess the accuracy of MRDTs were sensitivity, specificity, positive and negative predictive values, The results of the ICT Pf (*P falciparum)* was compared against blood microcopy, the latter being considered as the gold standard. (9)

The key variables used to measure MRDT positive and negative outcomes were: days to recover and unresolved malaria (including symptoms of fever chills, sweating, headache, nausea or vomiting).

Data were managed using EPI data version 3.1 and analysed using STATA 8.1.

4.3.6 Analysis strategy

Mean and standard deviations, or median and inter-quartile ranges were used to describe continuous variables. The following continuous variables were transformed into categorical variable by grouping:

- parasitaemia ≤ 500/µl, 501-5000/ µl or > 5000/ µl;
- age ≤24 or >24 and
- temperature ≤37.5^{0}C or >37.5^{0}C.

To determine ICT Pf MRDT sensitivity and specificity by level of parasitaemia, the parasitaemic readings were stratified into three categories (≤ 500, 501-5000, >5000). Results of the stratified sensitivity and specificity were compared with the WHO acceptable levels of accuracy.

To determine clinical predictors of malaria, uni-variate analysis, followed by modeling (logistical regression) was performed.

For uni-variate analysis, each explanatory variable was separately cross-tabulated with the outcome (positive microcopy result). Significance was judged using the Pearson's chi squared ($\chi 2$) measure of association. Mantel-Haenszel (MH) odds ratios (OR) and 95% confidence intervals were used to determine the magnitude of association for single variable and bi-variable analysis. Logistic regression was performed to determine the main predictors of malaria. All variables of clinical importance or those that were significant on uni-variate analysis or thought to be a predictor of malaria were included in the logistic regression model. A forward fitting analysis model was used, fitting one explanatory variable at a time to determine the main predictors of malaria in this population. Variables were judged for significant association ($p<0.05$) with the exposure, by using the Likelihood Ratio Test.

4.3.7 Ethics

Ethical approval was obtained from the London School of Hygiene and Tropical Medicine, (reference: 5061, 22 November 2006), Limpopo Health and Social Welfare Research Committee (reference: 4/2/2) and the University of Limpopo Research Ethics and Publications Committee (MR 123/2006). Nursing sisters at the health centres and clinics were informed of the study after ethical approval and approval by the Head of Health in the Limpopo Province.

Informed consent was obtained from each participating patient (written or thumb-prints where patients were unable to sign). Anti-malarial treatment was provided to all patients with a positive MRDT. See Appendix 9, 10 and 11 respectively for patient information sheet and informed consent.

Patient information was kept confidential, locked in a cabinet and was accessible only to the principal investigator (DM).

4.3.8 Potential bias

Observer bias could have been a possibility in this study when the Microscopists read slides. MRDT end-users (nurses) and Microscopists were blinded to results of each method. Microscopists examined slides at sites that were different to the sites where the MRDTs were conducted.(79)

4.4 Results

4.4.1Description of the study population

A total of 405 participants were enrolled in this study, from 01 December 2006 until 30 June 2007. Table 3 describes selected characteristics of the study population. The median age of study participants was 24.5 years old, ranging from 1 to 81 years old, (n=396). More male participants (~56%) were recruited into the study compared to females. Approximately 47% (N=402) of participants presented with fever - the median temperature of the participants was 37.5 ^{0}C (n=402); 29% (n=394) of the participants presented with chills, whilst 84% (n=399) of the participants presented with headache. The differences in sample sizes for each variable were due to missing values.

Characteristic		N	n (%)
Age	Range	396	1-81yrs (9*)
	Median		24.5 yrs
Sex	Male	399	225(56)
	Female		174(44)
Temperature	Range	402	35-40^{0}C
	Median		37.5^{0}C (3*)
Presence of Fever		402	189 (47.01)
Presence of Chills		394	114 (29)
Presence of Sweating		394	112 (28)
Presence of Headache		399	337 (84)

Footnote: The totals for individual variables <405 was due to missing values; * = IQR

Table 3: Distribution of selected characteristics in the participants

4.4.2 MRDT findings

Of the 405 patients tested, 198 (49%) were positive by ICT Pf and 191 (47.16%) were positive by microscopy, see Table 4. The kappa statistic comparing the two microscopists results were 0.95%; p<0.001, indicating good reliability. There was 19 discordant microscopy results between the first and second microscopy readers, these were settled when a very experienced 3rd microscopist at the reference laboratory read the slides.

Type of test	Malaria Positive N (%)	Malaria Negative	Total
ICT Results	198 (49.0%)	207 (51.1)	405
Microscopy Results	191 (47.16)	214 (52.84)	405

Table 4: MRDT and microscopy results in the study population

Among the 191 patients positive for Pf on malaria microscopy, 190 were positive for ICT Pf test (P<0.001), this represented 1 false negative result by the ICT Pf test, (Table 5). Among the 214 patients negative for slide microscopy, 206 were negative by ICT Pf test and this represented 8 false positive results by ICT Pf test.

	Results of Microscopy		
ICT	*P. falciparum* positive	*P. falciparum* negative	totals
P.falciparum positive	190	8	198
P. falciparum negative	1	206	207
Total	191	214	405

Table 5: Microscopy versus ICT results

4.4.3 Sensitivity and Specificity of ICT Pf Test

The overall sensitivity of the ICT Pf malaria test was 99.48%; P<0.001 (99% CI 96.17-100%; p<0.001), whilst the specificity was 96.26% (99% CI 94.7-100%; P<0.001), the positive predictive value of the test was 98.48 (99% CI 98.41-100.00%; p<0.001) and the negative predictive value for the test was 96.26% (99% CI 91.53-98.79; P<0.001). The J index for the test was 0.98; P<0.001 and the LRT test was 24.75-postive and 0.01-negative.

Sensitivity and specificity was calculated for three categories of parasitaemia (500, 501-5000; >5000 parasites/ µl of blood), in an attempt to determine the threshold for ICT detection. The sample size was too small for this analysis because parasite counts were only determined for 61 slides as thick films were not always correctly prepared by nursing staff, in spite of 2 follow-up visits conducted by the microscopists and the Principle Investigator (student). Furthermore there was only one false negative ICT Pf result and therefore there were no substantial observations in this analysis. The median parasitaemia calculated among the 61 slides were 25 680 parasites per µl of blood, ranging from 440 to >20 000 parasites per µl of blood. The sensitivity was 100% for all categories, see Table 6.

Parasitaemia (parasites/ µl of blood)	Microscopy No. of specimens	No positive	Sensitivity 95% CI	Specificity (95% CI)	PPV (95% CI)
0-500	2	2	100.00% (7.07-100.00%)	-	100.00% (7.07-100.00%)
501-5000	15	15	100.00% (0.50-100.00)	-	100.00% (0.50 100.00)
>5001	44	44	100.00% (88.66-100.00)	-	100.00% (88.66-100.00)

Table 6: Sensitivity and specificity of MRDT by levels of parasitaemia.

4.4.4 Clinical predictors of malaria

It was interesting to note that study participants positive for malaria were mainly older that 16 years of age (Table 7), however this finding was not statistically significant (p=0.2). In this study population there was a significant association between gender and malaria. The proportion of male participants having malaria was higher than female patients (56.0% vs 36.8.0%; p<0.001). The odds of malaria were approximately 2 times (95% CI 1.44 - 3.30, p < 0.001) more likely among the males compared to the females (Table 7).

A higher proportion of participants reporting a history of fever had malaria than those not having a history of fever (68.8% vs 27.7%; p<0.001). The odds of participants having malaria (microscopy slide positive Pf) was 5.6 times higher
95% CI 3.6 – 9.2, p<0.001) in those having a history of fever than those who did not have a history of fever (Table 7).

Participants having a temperature of >37.5 ^{0}C were more likely to have a slide positive malaria than those had a fever ≤ 37.5^{0}C. In fact the odds of malaria (slide positive Pf) in patients with a temperature > 37.5^{0}C was 4 times higher (95% CI 2.5-

6.3; P <0.001) than in those who had temperatures ≥ 37.5^{0}C than those whom had temperatures of less than ≤ 37.5^{0}C (Table 7).

Participants reporting chills, sweating or headache had a higher likelihood of malaria than those that did not report these symptoms (Table 7): The odds of malaria (slide positive Pf) in patients with chills were 4.7 times (95% CI 2.8-7.9; P <0.001) higher than in patients who did not have chills. The odds of malaria in patients with sweating were 8.9 times (95% CI 4.9 -16.3; P <0.001) higher than the odds in patients who did not have sweating. The odds of malaria in patients who had headache were 5.1 times (95% CI 2.4 - 10.3; P <0.001), higher than those patients without headache.

4.4.5 Modeling

Bivariate and multivariate analysis was used to determine the symptoms that best predicted malaria in the study population, whilst controlling for other variables. Sweating was considered to be a key predictor because it had the highest magnitude of association (OR) in uni-variate analysis and is one of the self-reported symptoms of malaria. The remaining predictors were fitted in the model in the following order: fever, headache, chills, temperature, sex and age. All the variables were serially fitted into the model. Age and chills were retained, despite it not being statistically significant as they were a priori predictors of malaria (as stipulated in the South African Malaria Treatment Guidelines).(8)

It was postulated that there was an interaction between sweating and fever i.e. that the relationship between sweating and malaria varies depending on the presence or absence of fever. Furthermore, that in the presence of fever sweating was more strongly associated with malaria (higher OR). In the absence of fever, sweating was due to another cause and was not associated with malaria. An interaction variable (sweating*fever) was added to the model in view of the above hypothesis, but this was found not to be statistically significant, and did not significantly change any of the ORs. Similarly there was no interaction between fever and headache, i.e. the

relationship between headache and malaria did not vary depending on the presence or absence of fever.

After fitting all the variables into the model sweating was considered to be the strongest predictor of malaria in the study population (OR 5.0; $p<0.001$; 95% CI 2.4-10.2). See Table 8 for the details of the regression analysis findings.

Predictors of malaria		Microscopy positive for malaria	Microscopy negative for malaria	Odds Ratio 95% (CI)	Adjusted Odds Ratio* (95% CI) (Final model)
Age	<5 years	10(34.5)	19 (65.2)	1.0	1.0
	6-15 years	37 (58.7)	26 (41.3)		
	16-49 years	131 (47.1)	147 (52.9)		
	≥ 50 years	11(42.0)	15 (58.0)	1.8 (0.8-4.0)	1.0 (0.4-.3.1)
Sex	Female	64 (36.8)	110 (63.2)	1.0	1.0
	Male	126 (56)	99 (44.0)	2.2 (1.4-3.3)	2.4 (1.4-4.1)
Fever	No	59 (27.7)	154 (72.3)	1.0	1.0
	Yes	130 (68.8)	59 (31.2)	5.6 (3.6-9.2)	2.9 (1.5-5.5)
Temperature	<37.5^{0}C	62 (31.2)	137 (68.8)	1.0	1.0
	≥37.5-39^{0}C	105(61.7)	65 (38.3)		
	>39^{0}C	19 (86.4)	3 (13.6)	4.0 (2.5-6.3)	2.1 (1.1-3.7)
Chills	No	100(31.0)	179 (69)	1.0	1.0
	Yes	82 (72.8)	31(27.2)	4.7 (2.8-7.9)	1.2 (0.6-2.4)
Sweating	No	93 (32.6)	190 (67.4)	1.0	1.0
	Yes	91(81.2)	21 (18.8)	8.9 (4.9-16.3)	5.0 (2.4-10.2)
Headache	No	11 (17.7)	51 (82.3)	1.0	1.0
	Yes	176 (52.3)	161 (45.7)	5.1(2.4-10.3)	4.0 (1.6-9.7)

Footnote: *Odds ratio of relationship between sweating and malaria adjusted for age, sex, fever, temperature, chills, and headache

Table 7: Crude and adjusted odds ratio for all predictors for malaria

	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Sweating Yes vs no	8.94 (4.92-16.2) p<0.001	5.3 (3.0-9.48) p<0.001	4.5 (2.47- 8.2) p<0.001	4.2 (2.2-8.2) p<0.001	5.2 (2.8-9.9) p<0.001	4.8 (2.3-9.8) p<0.001	5.0 (2.4-10.2) p<0.001
Fever Yes vs no		3.4 (2.1-5.5) p<0.001	3.7 2.2-6.0 p<0.001	3.4 (2.0-5.8) p <0.001	2.7 (1.5-4.8) p<0.001	2.9 (1.5-5.2) p<0.001	2.9 (1.5-5.5) p<0.001
Headache Yes vs no			3.4 (1.6-7.1) p<0.001	3.4 (1.6-7.1) p<0.001	3.7 (1.7-8.08) p<0.001	4.1 (1.8-9.5) p<0.001	4.0 (1.6-9.7) p<0.001
Chills Yes vs no				1.2 (0.67-2.4) p=0.45	1.1 (0.5-2.2) p=0.7	1.2 (0.6-2.4) p=.06	1.2 (0.6-2.4) p=0.6
Temperature ≥37.5^{0}C vs <37.5^{0}C					1.9 (1.1-3.3) p=0.02	2.0 (1.2-3.7) p=0.01	2.1 (1.1-3.7) p<0.001
Sex Male vs female						2.5 (1.5-4.2) p<0.001	2.4 (1.4-4.1) p<0.001
Age ≥6 years vs <5							1.0 (0.4-.3.1) p=.0.06

Table 8: Multivariate analysis for key predictors of malaria

4.4.6 Sensitivity and specificity for predicting malaria

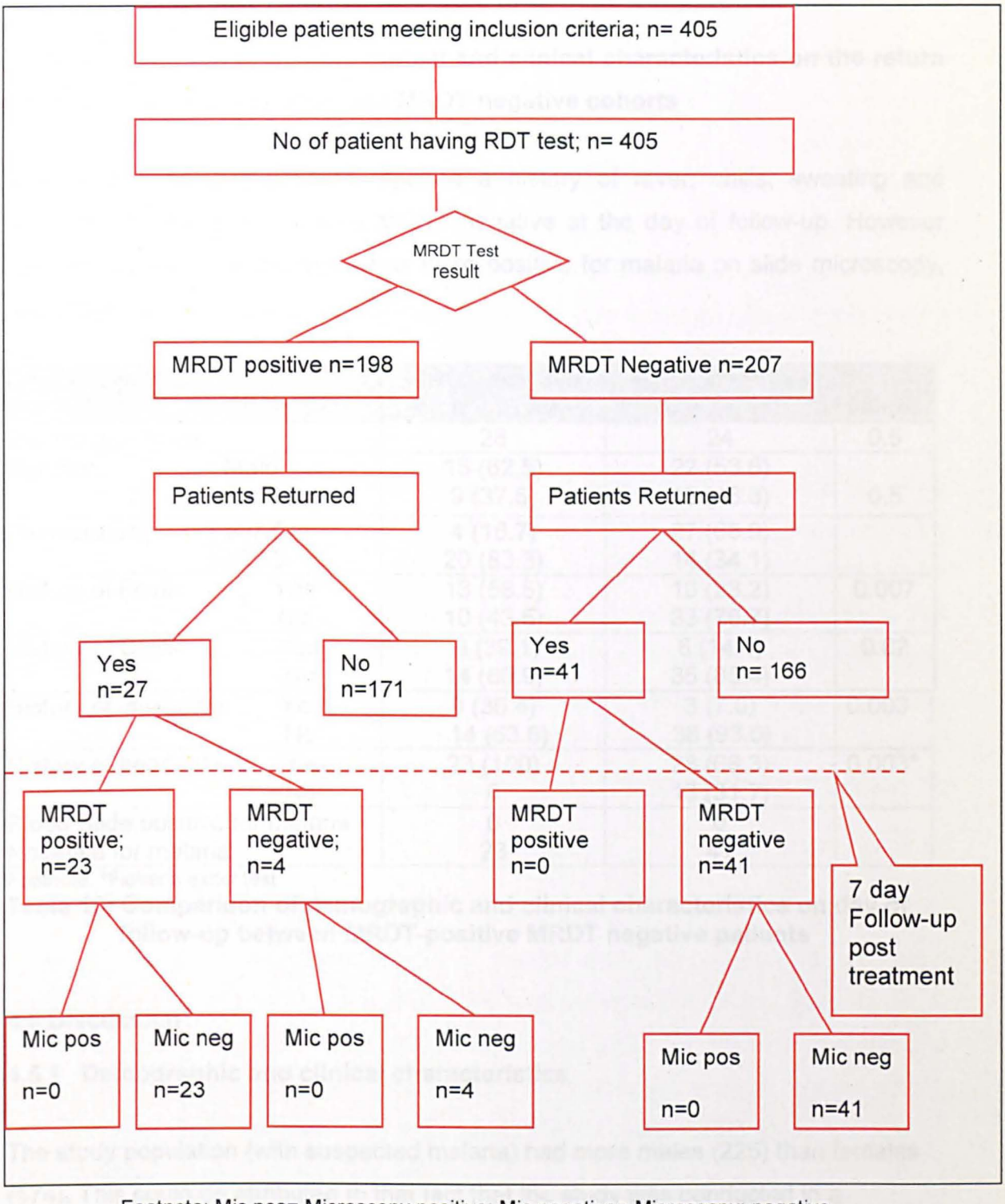
The overall sensitivity, specificity and positive predictive values of individual symptoms for predicting *P. falciparum* malaria (using microscopy) are summarized in Table 9. Sweating had the highest sensitivity followed by chills, then fever (using temperatures >37.5^0 C) and then headache.

Presenting symptoms	Malaria case N(%)	Not malaria N(%)	% Sensitivity (99%CI; Lower-Upper)	% Specificity (99%CI; Lower-Upper)	% PPV (99%CI; Lower-Upper)	% NPV (99%CI; Lower-Upper)
Sweating N=394	91 (47.6)	21 (9.0)	81.2 (70.0-89.7)	67.30 (59.7 – 74.7)	49.7 (40.0-59.4)	90.0 (83.5-94.6)
Chills N=394	82 (43.0)	100(47.0)	72.8 (60.8-82.8)	63.9 (56.1 – 71.2)	45.1 (36.6-54.8)	85.9 (77.9-90.9)
Headache N= 399	176 (92.1)	62 (29.0)	52.2 (45.1-59.3)	82.3 (66.7 – 92.7)	94.1 (88.2-97.6)	24.0 (16.9-32.4)
Temperature >38 °C vs 35-37 N=391	124(64.0)	68(31.0)	64.6 (55.1-73.3)	68.8 (59.7 – 77.01)	66.7 (57.1-75.3)	66.8 (57.8-75.0)

Table 9: Sensitivity and specificity of reported symptoms for diagnosing malaria (positive using slide microscopy) among patients attending health facilities of Mulala and Madimbo clinics of the Vhembe districts

4.4.7 Patient follow-up

Approximately 17% (68/405) of all the patients returned to the clinics for follow-up. All 41 patients who were ICT negative on initial visit remained ICT negative and microscopy negative on their return visit. Twenty three of the 27 patients who were ICT positive on the initial visit remained ICT positive on their return visit, however all 27 were microscopy negative, see Figure 8. All the patients who returned and were positive for ICT (n=23), recovered, none were sick and none had been hospitalized.



Footnote: Mic pos= Microscopy positive; Mic negative= Microscopy: negative

Fig 8: Patient follow-up with ICT and RDT results

4.4.8 Comparison of demographical and clinical characteristics on the return day between MRDT-positive and MRDT-negative cohorts

More MRDT positive patients reported a history of fever, chills, sweating and headache than those that were MRDT negative at the day of follow-up. However none of the MRDT positive patients were positive for malaria on slide microscopy, see Table 10.

Characteristics		MRDT positive at day 7 n=23	MRDT negative day 7 n=41	P value
Median age in yrs		28	24	0.5
Gender:	Male Female	15 (62.5) 9 (37.5)	22 (53.6) 19 (46.3)	0.5
Temperature	≥37.5 <37.5	4 (16.7) 20 (83.3)	27 (65.9) 14 (34.1)	
History of Fever	Yes No	13 (56.5) 10 (43.5)	10 (23.2) 33 (76.7)	0.007
History of Chills	Yes No	9 (39.1) 14 (60.9)	6 (14.6) 35 (85.4)	0.02
History of sweating	Yes No	8 (36.4) 14 (63.6)	3 (7.0) 38 (93.0)	0.003
History of headache	Yes No	23 (100) 0	28 (68.3) 13 (31.7)	0.003*
Blood Slide positive for malaria Negative for malaria		0 23	0 41	

Footnote: *Fisher's exact test

Table 10: Comparison of demographic and clinical characteristics on day of follow-up between MRDT-positive MRDT negative patients

4.5 Discussion

4.5.1 Demographic and clinical characteristics

The study population (with suspected malaria) had more males (225) than females (174). This could be attributed to that fact that the study was conducted in a population that includes migrant Labourers from surrounding malaria-endemic countries doing manual work on farms in the Limpopo province; hence more males take on these types of jobs. The prevalence of malaria was also highest amongst males than females. This is probably because the manual labour performed by most

males would expose them to mosquitoes especially at dawn and dusk periods where the mosquito vector are most active.(76)

The national treatment guidelines for malaria in South Africa stipulate that clinical judgment should be applied in the absence of malaria diagnostic tests.(8) The WHO stipulates that when there is a high clinical index of suspicion but a negative malaria diagnostic test, treatment for malaria should be administered whilst confirmatory diagnosis is being pursued.(9) Predictors for malaria therefore become very important if clinical judgments are to be made.

The IMCI guideline for management of childhood illness in South Africa (used for clinical diagnosis of malaria in children age five years and below - before diagnosis with RDTs) stipulates that fever (temperature $\geq 37.5^0$C) is the main predictor for malaria.(82) Temperature $\geq 37.5^0$C was not the main predictor of malaria in this study population. Sweating and headache were much stronger predictors of malaria than elevated temperature in this study population. Although the sample is not representative of the facilities of the Vhembe district, data from this study suggest that symptoms such as sweating, headache and chills should be considered for clinical judgement of malaria.

All the signs and symptoms (tiredness, myalgia, abdominal pain, diarrhoea, loss of appetite, nausea, vomiting and cough) of malaria were not recorded in this study, hence a comprehensive judgement of them was not possible for the analysis. (8) A study should therefore be conducted to determine which of the clinical signs and symptoms of malaria would be key predictors of malaria in the South African setting and a clinical algorithm should then be generated to guide health care workers on making clinical discussions.(8, 83, 84)

A clinical algorithm should be considered in the South Africa setting when there is a high clinical index of suspicion of malaria and slide microscopy or MRDTs are unavailable or MRDT is negative, especially at health care facilities are far away from secondary care hospitals.

4.5.2 Agreement between microscopists

There was reasonable agreement in the slide reading by the 2 microscopists (kappa=0.95). However an analysis of the 5% of the discordant microscopy results between the first and 2nd microscopist showed that 94% (n=19) of the first microscopists readings were false negatives and 6% false positive. Parasite counts were only conducted in approximately 52% (10/19) of the discordant results. Parasite counts could not be confirmed on nine slides as the slides were poorly stained or prepared. However those slides that did have parasites counts showed high level parasitaemias (range: 560 - > 20 000 parasites/ µl of blood) by the second and third readers, implying that these were missed by microscopist one. Some studies have found that the sensitivity of microscopy starts to decline with low level parasitaemias <100 parasites / µl of blood (67, 85, 86); however in the current study most of the malaria positive slides that were missed had >100 parasites per µl of blood. This findings, begs the question of whether microscopy quality within the malaria affected areas of the Limpopo are adequate for diagnosis of malaria. An assessment of proficiency of microscopy within the malaria affected provinces should be considered by the health authorities, should there be a deficiency in the quality of microscopy then action would need to be taken to remedy this service. s

4.5.3 Sensitivity ICT Pf test

The overall sensitivity of the ICT Pf in this study was high viz. 99.48, P<0.001 these findings are consistent with that of other ICT Pf studies.(37, 38) However it was not possible to assess the MRDT sensitivity at a parasite density of 100 parasites / µl of blood, in accordance with WHO criteria for judging the sensitivity of an RDT.(9, 23) This was due to the fact that malaria parasitaemia levels were assessed only on 61 slides due to poor quality staining and poor slide preparation by nursing staff. Among the 61 slides assessed for parasitaemic levels all the slides had more than 100 parasites per µl of blood. The sensitivity was 100% from all categories of parasitaemias >100 µl of blood in the study. Whilst low level parasitaemias may have been picked up if all the slides had parasite counts, information from the malaria programme (personal communication malaria manager Limpopo province)

suggests that patients presenting to health facilities present late; thus few would have had low levels of parasitaemia. Assuming this was true, then low levels parasitaemic patients will be very few even if there was parasite counts in all participants. This would then make the current estimation of the parasite detection level to be reasonable.

An alternative approach to determining parasite detection levels by the ICT Pf MRDT is to use laboratory dilution of wild type parasites, similar to that conducted by other authors.(56) The Medical Research Council of South Africa evaluated the ICT MRDT tested in this study in the laboratory, when this study was in progress, for parasite detection limit, using the protocol by Craig et al. (87) The results from the laboratory studies showed that the ICT Pf test gave 100% sensitivity at a parasite density of 70 parasites per µl of blood.(9) Although laboratory investigations is not ideal for determining MRDT parasite detection limit, the MRC's finding does however give some indication of ICT Pf sensitivity and specificity at low parasite densities.(9, 22, 39).

The overall results of the ICT test showed that there were one false negative result from the ICT test and 8 false positive results. These findings are discussed below:

4.5.4 False negatives

The false negative rate is defined as, the proportion of infected individuals being missed by the ICT Pf test and falsely ascribed a negative status. (9, 22, 39)
According to the published literature there could be a series of reasons for the false negative results, these include:

- reduced level of parasites in circulating blood or reduced sensitivity of microscopy at low parasite levels, (40, 44, 88-90)
- decreased antigenaemia post treatment of patients, (28, 44, 91-95)
- probable poor end-user interpretation of weak positive results, (93)
- weak levels of antigens at early stages of infection (36) and

- negative results in view of sexual stages of the parasites. (52)

The only false negative case seen in this study was in a slide that had 1 920 parasites per/ µl of blood and the patient was not on treatment for malaria. Other studies have also reported similar findings where, false negative results did occur with high parasitaemias.(42, 96-98) Wongsrichanalai et al. from a study in Thailand, when comparing ICT RDTs with microscopy, found that they had one false negative MRDT result in a patient that had a parasitaemia of 18 000 parasites per µl of blood.(95) Similarly Forney et al. found in a multi-site field study in Peru and in Thailand noted that the parasite density ranged from 201- >5000 parasites/µl of blood among 9 patients who had false negative result when tested with parasite F test.(96) The median parasitaemia levels in these patients were 16 and 203 parasites/µl of blood in the 2 respective studies.

Whilst one cannot be certain of the reasons for the false negative results in this study by ICT Pf, in the presence of a high level parasitaemia, some possible explanations could be:

- that there was HRP II antigen variation in the blood sample from those designed to be captured by the monoclonal antibodies of the ICT Pf testing system.(99)
- that anti-HRPII-Pf antibody potentially blocked immuno-detection by the ICT Pf testing system. (100, 101)
- antigen accelerating HRPII-Pf clearance occurred.(95)

Whilst MRDT false negative results were rare in this study, health workers should remember that a negative result reduces the chances of, but does not absolutely rule out the likelihood of malaria. Furthermore false negative rates are likely to increase if the MRDTs are stored for longer periods in field conditions.

4.5.5 False positive results

The false positive rate was calculated as the proportion of individuals without infection being missed by the test and falsely ascribed a positive status.(9, 39)

There were eight false positive ICT Pf results in this study. Some of the reasons for the false positive results could be due to the following:

- patients previously treated for malaria, could still having circulating antibodies which can persist for weeks after treatment as seen in other studies.(35, 40, 42) It was unlikely in this study for patients presenting to clinics as first time visitors after receiving malaria treatment as it was standard practice for patients to be followed up by slide microcopy at the health centers. Secondly patients who had recent malaria or had recently been on malaria treatment were excluded from the study.
- episodes of recent fever could also produce false-positive results due to high levels of circulating non specific heterophile antibodies.(102)
- rheumatoid factor could cause false positive results with some RDTs. The effect of rheumatoid factor was not measured in this study, however according to the manufacturers package insert, rheumatoid factor has not been shown to give false positive results.(103)
- RDT can yield false positive results in patients with *P. falciparum* malaria where the parasites are sequestered out of the patient's circulation, such as during pregnancy.(67) In this study it is unlikely that this occurred, as none of the patients whom were recruited in the study were recorded as being pregnant. According to the national malaria treatment guidelines in South Africa, all patients whom are pregnant with malaria should be referred to hospital, further obviating the reason for false positive results with ICT Pf in this study.(8)

A highly sensitive test such as the ICT Pf test as seen in other studies and in this study will yield lower specificities, especially in patients with low parasitaemias.

(38, 67) Whilst microscopy can detect low level parasitaemias (10-50µl) in specialized centres, microscopy of blood films in malaria endemic areas has been shown in other studies to miss cases with low density parasitaemias.(104) The quality of microscopy and the slides prepared in this study was not optimal, hence lower density parasitaemias could have been missed by slide microscopy questioning the gold standard and making this the most likely reason for false positive ICT Pf results in the study.

4.5.6 Specificity

Specificity is the ability of the test to detect individuals without infection as negative. (9, 39) The specificity of the test was 96.26%. The relatively lower specificity than sensitivity, in this study is similar to other studies.(37, 40, 48) Having a relatively lower specificity which leads to over-diagnosis and to over treatment of non malaria cases (19) is considered less serious than having a lower sensitivity.(38) The low specificity could possibility be explained by poor quality slides resulting in lowering of the sensitivity of microscopy, or very low parasite densities that were undetectable by microscopy.(9, 67)

4.5.7 Positive predictive values

Positive predictive value of the ICT Pf test is defined as: the proportion of the test's positive readings which are truly positive.(9, 39) The positive predictive value of the ICT Pf malaria test was 98.48%. The false positive rate of the test contributed to a lowering positive predictive value of the test. However a positive test in the study setting contributed to having reliably diagnosed malaria parasites with a low risk of error.(38) The Positive Predictive Values of a test correlates with prevalence of disease in the population being tested.(79) Compared with low transmission settings, in high transmission settings positive predictive values of the ICT pf test are usually lower (because of the higher prevalence and larger numbers of false positives, larger denominator and smaller numerator) (32, 33) In low transmission settings positive predictive value are usually high.(26, 28) In this study the incidence of malaria was low - 3.28 per 1000 population at risk - and the positive predictive

value of the ICT pf test was high, with good accuracy. Whilst every attempt should be made to undertake feld evaluations on MRDTs the findings of this thesis imply that other malarious provinces within SA and countries with similar malaria transmission as South Africa can adopt or consider adopting ICT pf as a diagnostic test for malaria.

4.5.8 Negative predictive values (NPV)

The negative predictive value of a test is the proportion of negative readings which are truly negative. In the study the negative predictive value was 96.26%, $p<0.001$. The risk of missing a negative case in the study setting is very small. This high negative predictive value allows health workers to confidentially exclude malaria if the ICT test was negative. These findings correlate well with low prevalence malaria transmission settings, making it an ideal test for implementation in similar settings.(26, 28)

4.5.9 J Index

The J index of the test is the overall measure of reliability of the diagnostic test which summarizes both sensitivity and specificity. The J index lies between 0 and 1 - when the J-index approaches 1, the overall diagnostic ability of the test approaches the ideal level.(39, 105) The J index of the ICT Pf test was 0.98, making the test ideal for malaria diagnosis in this study setting.

4.5.10 LRT test

The positive likelihood ratio was 24.75, this is indicative that a positive rapid test result implies a high probability to having disease, due to the likelihood ratio being >10. Conversely the negative likelihood ratio of 0.01 implies that test excludes malaria when it is negative.(106)

4.5.11 Patient follow-up

The main reasons hypothesized for the low turn out patients was that they were likely to be illegal immigrants possibly from Zimbabwe (close proximity to the clinics) and that that many could have been arrested and repatriated, others may have not returned home or were too scared to return to the clinic when they felt better (personal communication Provincial Malaria manager Limpopo).

ICT positive results in returning patients that were positive for malaria is consistent with other studies where HRP II takes weeks to disappear.(9, 106, 107)s This finding is consistent in that ICT Pf test can only be used as a screening test and not a test to monitor treatment. The South African malaria treatment guideline for patient follow-up is therefore justified in stating that patients should be followed up with slide microscopy after being diagnosed with RDTs.

The favourable clinical outcomes for ICT positive patients are an indication of parasitological cure. However as the numbers for the returning of patients were small it would be difficult to determine the numbers of those patients whom were misdiagnosed (false negatives) and went on to developing malaria.

It is not known how many patients who did not return for their follow-up visit had been hospitalised and how many had died. Attempts were made to trace patients in the local hospital, and to peruse death certificates at the local housing office, but none of the patients lost to follow-up were found. In view of this high loss to follow-up, it is likely that the follow-up do not reflect the true outcomes amongst ICT-positive and ICT-negative patients. Therefore follow-up data should be interpreted with caution and conclusions cannot be drawn from them.

The more symptoms of malaria reported in the MRDT positive cohort versus the negative cohort on day of return could have been due to malaria patients recall bias.

4.6 Conclusions

The study revealed that the ICT Pf test is an appropriate test to use in the field, where laboratory facilities are not available. The test has a high degree of sensitivity and acceptable level of specificity against the WHO criteria. Sensitivities could however, not be established at low levels of parasitaemia, viz. <100 parasites/µl of blood in field conditions. The low parasite detection limit (100% sensitivity at 70 parasites/ µl of blood by the ICT Pf at the laboratory setting) suggests that ICT Pf test is appropriate for use in South Africa.

The ICT Pf can be reliably used as screening test for diagnosis of *P. falciparum malaria,* the test however, cannot be used for patient follow-up and for monitoring therapeutic responses due to the persistence of HRPII positive results post treatment.

Sweating and headache were stronger predictors of malaria than a history of fever and these symptoms merits consideration for inclusion in the IMCI guidelines for management of febrile illness in children.

5.0 Chapter 5: MRDT end-user proficiency study

5.1 Introduction

One of the factors that can affect the outcome of the MRDT result is end-user proficiency i.e. the ability of the end-user to perform the MRDT and interpret the results.(9, 22, 59, 60, 62) As stated in chapter 2, MRDTs have been implemented for malaria diagnosis in South Africa since 2001; however the level to which end-users were proficient in MRDT use has not been determined.(3, 7)
This study was therefore conducted to assess the end-user proficiency in the Vhembe district in the Limpopo Province of South Africa.

5.2 Objectives

The key objectives of the study were to determine the end-user's ability to:
(1) prepare the MRDTs;
(2) perform the MRDTs and
(3) interpret the MRDT results

5.3 Methods

This was a cross sectional observational study to determine whether health workers performed the MRDTs and interpreted the results correctly at one point in time.

The study was conducted among professional nursing staff that were responsible for using MRDTs in clinics and health centers of the Vhembe District. Based on the average annual malaria cases reported from 2004 to 2006, all health facilities (clinics and health centres N=50) were classified into low transmission (10-19 malaria cases per year), medium transmission (20-50 cases per year) and high transmission (>50 malaria cases per year). Fifty percent of clinics and health centres (n=25) were randomly selected from each group (11/22 from low; 5/10 from medium, and 9/18 from high transmission health facilities). From each selected health facility one

MRDT end-user was selected using random numbers, (see Appendix 12 for clinics and health centres selected). Data on demographics and professional experience were collected from all selected end-users. The participants' ability to perform the MRDT test was assessed using a non-patient volunteer (known MRDT negative person). A checklist of observations, adapted from previously studies and information extracted from the manufacturers package insert was developed by the student.(9, 63) See Appendix 13, for the checklist on end-user ability to perform MRDTs on a non-patient volunteer. The checklist included the end-users' ability to prepare the volunteer for collecting blood sample using sterile techniques, and to perform the test according to the manufacturer's instructions.

The end-users abilities to interpret the MRDT results were assessed using photographs of pre-prepared RDT results (see Appendix 14). In total there were ten photos – 4 strong positive, 4 weak positive, 1 negative and 1 ambiguous result (no control line with test line positive). All end users were shown the same photographs, and in total end-users should have interpreted 200 photographs as being positive (25 end-users interpreting 8 positive photographs), 25 as being negative and 25 as being invalid. Using the pre-prepared test results as the gold standard the sensitivity, specificity and the predictive values of the end-users' interpretation of the test results was calculated.

The main outcome measures for the study analysis were performance score and interpretation score. Performance score measured the extent to which end-users performed all the 15 steps when doing a rapid test. A score of 1 was allocated if each of the 15 steps were correctly performed and a score of 0 was allocated if each step was not or incorrectly performed. The maximum performance score was 15. Thus the performance score of each end-user (out of 15) measured the extent to which they followed 15 essential steps when doing the MRDT.

The interpretation score was developed by determining the extent to which end-users correctly interpreted the photographs of the battery of ten pre-pared MRDT results. A score of 1 was allocated each time a test was correctly interpreted and a

score of 0 was allocated each time a test was incorrectly interpreted. Thus the maximum interpretation score was ten (for then correctly interpreted results). The interpretation score measured the extent to which the end-users correctly interpreted the results of photos of 10 prepared MRDTs.

5.3.1 Data analysis strategy

Data checking and editing to identify missing data and errors was performed by examining frequencies and cross tabulation for categorical variables. Range checks were used for continuous variables, to identify values falling outside the expected ranges. Histograms were also used to identify outliers that looked extreme relative to the rest of the data.(71, 81)

During data analysis, frequencies of various participant characteristics viz: socio-demographic; educational level; treatment experience; MRDT experience training with MRDTs; clinic; test type and performance scores were run. Variables were re-categorised to dichotomize the data these include participant age; performance scores and interpretation scores. Means and standard deviations were used to describe normally distributed data whilst median and inter-quartile range was used for non-parametrically distributed data.

The performance scores were dichotomized based on the median scores and uni-variate analyses were conducted to determine the association between the performance score and the following variables:

- the type of test used (ICT or Makromed);
- clinic cases (low, medium, high);
- participant's age;
- training on use of MRDT and
- experience.

The median was used as a cut off instead of another critical score as it was believed that each step in the checklist used to observe end-users was of equal importance, non-performance of any one step could have deleterious consequences; thus no step or combination of steps was more critical than others. Furthermore it was equally important to correctly interpret each test from the battery of pre-prepared tests, thus dichotomization based on the median yielded an adequate sample size in each category to conduct further analysis and to compare groups.

Mantel-Haenzel odd ratios and Pearson's Chi-Square (X^2) tests were used to determine the association between key categorical variables and a p value of <0.05 was regarded as statistically significant.

The validity of end-users interpretation of the 200 positive, 25 negative and 25 ambiguous results were assessed by examining the sensitivity and specificity of their interpretation of MRDT results. The reliability of end-users was tested by determining the absolute agreement- interclass correlation coefficient for a two-way random model (as the end-users – participants- were randomly selected). It was thought that this analysis would be useful to compare the mean interpretation score for each participant (which ideally should be the same), and determine the extent to which they absolutely agreed, and to identify those participants that need retraining as they reduce the intra-class correlation coefficient, and thus the reliability.

5.4 Ethics

Informed consent (written) was obtained from each participant (See Appendix 15 and 16). Participant information was kept confidential, locked in a cabinet and accessible only to the Principle Investigator. Participants were and will not be identified in any reports or communications.

5.5 Results

5.5.1 Characteristics of the participants

The total number of participants in this study was 25 (5 male and 20 female); 18 were nurses and seven were nursing assistants (Table 11). The median age of the participants was 40 (IQR=10). All nurses included in the study had experience in malaria diagnosis and treatment, and their mean years of experience were 5 years. The 7 nursing assistants had experience in malaria diagnosis but not treatment. This is in keeping with current South African malaria policy that only professional nurses are allowed to treat patients.(8) All the study participants had experience in using a MRDT with a median of five years (IQR) experience. However only 24% (6 of 25) received external training on MRDT use through the malaria control programme - the remaining 76% received in-house training from colleagues at their health facility. Thirty two percent of the clinics used (eight of 25) ICT diagnostic test whilst the remaining 68% (17/25) used the Makromed. This was because ICT tests were still being introduced to the clinics at the time of this study, whilst Makromed was being phased out. This provided a unique opportunity to compare end-users proficiency on each test.

Characteristic	**N (%)**
Total number of participants	**25**
Age in years Median [IQR]	40 [10]
Gender Male Female	 5 (20) 20 (80)
Qualification Nurse Nursing assistant	 18 (72) 7 (28)
Has previous experience in treating malaria experience	18 (72)
Has used Malaria RDT Mean years experience in using of RDT use	25 (100) 4.4 (SD=1)
Has received training on MRDTs	6 (24)

Table 11: Participant characteristics

5.5.2 End- user proficiency: individual steps

None of the participants completed all steps successfully; a median score of 11 out of a total of 15 was obtained by end-users to correctly perform the MRDTs. The end-user performance on each step is summarized in Table 12.

5.5.3 Recording patient information

Ninety two percent of the participants did not write the patients name on the test cassettes, however 72% participants entered information in a patient's register.

5.5.4 Preparing MRDT before the conducting the test

Although 80% of the participants were able to assemble the test kit, 16 (64%) participants did not check the expiry dates on the test packages.

5.5.5 Using sterile procedures to conduct the test

Sixty eight percent of participants did not use gloves when they performed the test and 20% of the participants did not clean the volunteer's finger with an alcohol swab.

5.5.6 Adherence to test procedures

Seventy six percent (19 of 25 end-users) used the lancet enclosed in the kit whilst the remaining 24% used sterile clinical needles to prick the volunteer's finger. The reasons given by the participants for this practice were that they did not get sufficient blood for performing the test, when they used the lancet from the kits. Ninety two percent of the participants added the blood to the correct window, although 16% did not use the correct amount.

Sixty eight percent of participants used the correct amount of buffer to conduct the test; the remaining 32% each used 3 drops instead of the 6 for Makromed test-kit. Seventy six percent of the participants waited 15 minutes to read the test, the remaining 24% each waited 3 minutes only.

5.5.7 Interpretation of actual test result

Seventy six percent of participants correctly read MRDT results (performed on the known MRDT negative volunteer), however the remaining 34% read the results as

being ambiguous. Interestingly, all 34% participants were those who used sterile needles for pricking the finger of the volunteer instead of the lancet provided in the test kit.

Steps in Test procedure	N=25(%)
Assemble the new test cassette,	20 (80)
Put on a new pair of gloves	8 (32)
Check expiry date on the test package to make sure test is still valid	9 (36)
Write patient name on the device	2 (8)
Clean finger with alcohol swab	20 (80)
Allow finger to dry before pricking it	17 (68)
Using a sterile lancet, puncture the side of the ball of the finger	19 (76)
Dispose of lancet in sharps bin immediately after pricking finger	16 (64)
Touch one end of the sample applicator pipette (straw) to the blood on the finger prick for Makromed or draw up to the first line in the pipette for ICT	21 (84)
Using the pipette, immediately touch the tip of the pipette with the blood in Window A on the test device (smaller hole in case of ICT)	23 (92)
Dispense 6 drops of buffer (MAKROMED) or 5 drops of (ICT)	17 (68)
Wait 15 minutes before reading the results	19 (76)
Read test results correctly	19 (76)
Record results in register	18 (72)
Dispose gloves, wrappers, alcohol swab, loop, desiccant and cassette in non-sharps container	21 (84)

Table 12 Adherence to the test procedure by the participants

5.5.8 Interpretation of prepared test results

The interpretation results were grouped into the categories of: i) weak positive, ii) strong positive, iii) negative and iv) ambiguous. See Table 13 for a breakdown of the end-user interpretation of the scores.

The overall median interpretation test scores (calculated from a possible total score of 10 correct responses where a score of 1 was assigned if interpretation was correct and a score of 0 was assigned if interpretation was incorrect) was 9 (IQR=2). Seventy-eight out of a total of 100 weak positive MRDT results were correctly interpreted by the participants. From the remaining 22, 10 were interpreted, as negative and 12 as ambiguous. Ninety-nine of the 100 strong positives were interpreted as positive and 1 was interpreted as negative. Twenty-four of the 25 negative results were interpreted as negative and 1 was interpreted as ambiguous. Of the 25 ambiguous results, 15 were interpreted as ambiguous, 8 as negative and 2 as positive. The sensitivity[a] of end-users interpretation of positive test results was 85%. The specificity[b] of end-users MRDT result interpretation was 96% and the probability of correctly identifying ambiguous results was 60%.

True results	Participant's responses			
	Positive	Negative	Ambiguous	Total no. observations (n)
Weak Positive	78	10	12	**100**
Strong Positive	99	0	1	**100**
Negative	0	24	1	**25**
Ambiguous	2	8	15	**25**

Table 13: Agreement between the true results and the participants interpretation scores

5.5.9 Reliability analysis

Reliability analysis showed poor reliability of a single end-user (participant/ nurse) – ICC (2,1) of 0.54, and increased reliability when the scores of all end-users (participant/ nurse) were considered together. The results/interpretation of a single

[a] Probability that end-users correctly interpreted a result as being positive when it was truly positive
[b] Probability that end-users correctly interpreted a result as being negative, when it was truly negative.

end-user is not reliable and end-users cannot be interchanged. Data were interrogated to determine whether one particular end-user reduced the reliability. Analysis showed that end-users 2, 4, 7, 20, 21 and 24 and 25 varied in their interpretation of MRDTs reliability. Five of these seven end-users received no training on MRDTs, co-incidentally their mean years of working with MRDTs was 4.5 (SD=1.39). This finding highlights the need to address end-user training.

5.5.10 Frequencies of key variables against performance outcomes

Table 14 presents distribution of the performance and interpretation scores by the MRDT type, clinic transmission, age of participant, qualification of participant, training on MRDT and participant treatment experience.

The median participant scores for all two outcomes (performance and interpretation scores) were higher for the ICT MRDT than the Makromed ICT.

The performance scores were higher in the high malaria case load clinics (> 50 malaria cases per year) than the medium and low malaria transmission clinics. The median participant interpretation scores were lowest in the low malaria case load clinics (10 –19 Malaria cases per annum). The lowest median interpretation scores were in the nurse age category 40-59 years. The median performance scores and interpretation scores of the end-user were higher in qualified nursing staff than unqualified staff. Nurses who were trained on MRDT had slightly higher interpretation scores than those untrained. Nurses who had no treatment experience had slightly better performance scores than those who had treatment experience.

Characteristics of MRDT and participants		Median Performance score (IQR)	Median Interpretation score (IQR)
MRDT test type	ICT	11.5 (1.5)	9.5 (1.5)
	Makromed	10.0 (2.0)	9.0 (3.0)
Clinic transmission settings	Low transmission	10.0 (4.0)	7.0 (4.0)
	Medium Transmission	10 (3.5)	9 (2.0)
	High Transmission	11 (3.0)	9 (1.0)
Age of participants:	20-39	11(1.5)	9 (1.5)
	40-59	9 (4.0)	9 (2.0)
Nurses qualification	Qualified Nurse	11.0 (3.0)	10.0 (3.0)
	Nursing assistant	10.5 (4.0)	9 (0.0)
MRDT training	Yes	11.0(2.0)	10.0 (1.0)
	No	11.0 (4)	9.0 (3.0)
Treatment Experience	Yes	10.5 (3)	9.0 (3.0)
	No	11.0 (3)	9.0 (1.0)

Table 14: Distribution and performance and interpretation scores by type of MRDT and participants characteristics.

5.5.11 End-user performance of MRDTs

There was no significant association between performance scores and test type, age, MRDT training, qualification and clinic malaria risk- see Table 15.

Characteristics of MRDT and participants	Performance scores above the median	Odd Ratio [95% Conf. Interval]
Makromed n=17 ICT Pf n=8	3 (17.6) 4(50)	1 4.67 (0.61-35.18)
Low/medium case load clinics n=7 High case load clinics n=18	1(14.3) 6(33.3)	1 0.331 (0.03 -3.8)
Age 40-59 n=13 Age 20-39 n=12	3 (23.08) 4 (33.3)	1 0.6 (0.1-3.6)
No training n=19 Had MRDT training n=6	4 (21.0) 3 (50.0)	1 3.75 (0.4- 29.5)
No treatment Experience n=7 Had treatment Experience n=18	3 (42.9) 4(22.2)	1 0.38 (0.05- 2.6)
Nursing assistant n=10 Qualified Nurse n=15	2(20) 5 (33.3)	1 2.0 (0.29-14.0)

Table 15: Association between characteristics of MRDT/participants and the performance scores

5.5.12 Interpretation of MRDT results

Nurses trained in MRDT use had significantly better interpretation scores than untrained nurses (OR=2.9 95%CI 0.25-33.1; p=0.003). There was no significant association between interpretation scores and test type, low versus high transmission clinics, younger versus older nurses, nurses with treatment experience and those without and professional nurses versus staff nurses (Table 16).

Characteristics of MRDT and participants	Interpretation scores above the median	Odd Ratio [95% Conf. Interval]
Makromed n=8 ICT Pf n=17	6 (75.0) 11(65.7)	1 0.6 (0.1-3.3)
Medium/low case load clinics n=18 High case load clinics n=7	10(5.55) 7(100)	-
Age 40-59 n=13 Age 20-39 n=12	8 (61.6) 9 (75.0)	1 1.9 (0.2-14.3)
No training n=19 Had MRDT training n=6	12(63.16) 5 (83.3)	1 2.9 (2.5- 33.15)
No treatment experience n=7 had treatment experience n=18	6 (14.3) 11(61.1)	1 0.3 (0.02- 2.9)
Nursing assistant n=10 Qualified Nurse n=15	8 (80) 9 (60.0)	1 0.37(0.59-2.6)

Table 16: Association between the characteristics of MRDT/participants and the interpretation scores

5.6 Discussion

Although the sample in this study is small, it was carefully chosen using multi-stage stratified random sampling; this increases the likelihood that the study population is representative of end-users in the study area. The study has shown that a large percentage of end-users did not receive standardized training on MRDTs. Standardised training of the end-user is critical to addressing the weaknesses in end-user proficiency with the use of MRDTs.(9)

5.6.1 End-user performance

All the key activities viz; recording patient information, preparing the test kit, using correct sterile procedures, adhering to the test instructions and correctly interpreting the MRDT results can be improved, with a view to improving end-user performance and reducing malaria-related morbidity and mortality. One consequence of not using proper sterile procedures is the possibility of cross infection with other diseases. The Vhembe District is a high HIV risk district in South Africa, with HIV prevalence being 11.1% (108); thus poor adherence of end-users to sterile procedures is of grave concern. The use of too much blood on the MRDT test strip by some participants is cause for concern, as weak positives, in early stages of the infection could be overlooked.(9) This was corroborated by this study as data indicates that end-users who used too much blood interpreted the results as negative. The inadequate amounts of buffer used by some participants, could lead to poor clearing of the patient's blood across the test strip, this could cause false negative results.(67) The short time to read the MRDT as practiced by some participants could result in false negatives and have disastrous consequences for the patients, especially those that would be at the early stages of the infection with low levels of parasitaemia.(9, 66)

The tendency for performance scores to be better for ICT Pf than Makromed can be attributed to 2 key steps in the testing procedure. Firstly ICT Pf makes use of a specialized pipette, enabling end-users to draw up 5 µl of blood and a specialized dropper to deliver 5 drops of buffer. This prevented the nurses from making errors as they did with Makromed test-kit. The ICT Pf test had a higher odds ratio (4.67) for performance scores when compared with the Makromed test however the wide confidence intervals point to the study possibly having low power thus contributing to a statistically non significant finding (p=0.096).

The significantly better performance and interpretation scores of trained nurses compared to those untrained nurses (p=0.0038) shows that even though more

participants were trained in-house than using the standardized external training, they were more proficient than nurses receiving no training. Once again the wide confidence intervals point to insufficient power in the study, due to small sample size. This finding can be corroborated with similar findings in other studies that training can improve end-user proficiency. (9, 59, 62) Job instructions or job aids such as wall charts should be considered for implementation in the study setting and must make provision for pictures on weak positive and ambiguous results, similar to those in other studies.(9, 59, 62) It is crucial that end-users are trained prior to and during the supply of newly introduced MRDTs as this is likely to improve performance. (59, 60, 62)

5.6.2 Interpretation of the battery of tests

The 15% false negatives resulting mainly from the poor interpretation of the weak positive photographs, are concerning. The end-users probably expected the strength of the test line to be as strong as the strength of the control line in order to interpret the results as positive Bell et. al. state in their review on ensuring quality of diagnostics that weak positives occur during the early stages of malaria infections, thus determining and treating malaria at this stage is critical to preventing serious morbidity.(67) South Africa can be considered as a country with low level of malaria transmission, hence low level symptomatic parasitaemias are highly likely especially during the early stages of the infection.(3)

The findings on the interpretation of weak negative pre-prepared photographs are similar to that documented in other studies.(59, 60, 109) A study from Switzerland on volunteer's ability to interpret malaria RDT results showed a false negative interpretation rate of 72% of one test and 29.6 of another test – these tests were prepared from patients with low parasitaemias (<0.1% blood parasites).(60) Trachsler et. al. in another study from Switzerland, observed 160 participants for their ability to interpret 800 prepared malaria rapid test results.(62) Participants correctly interpreted 70.6%, there were no false positives however there was 14.1% false negatives, 6% of which were from weakly positive malaria rapid tests. (62)

Rennie et al., in a study from the Philippines, tested community health workers who performed and interpreted malaria rapid tests and interpret results ; they also used a battery of prepared results. (59) They found that the true positivity rate was between 63-66% from their 2 study groups; most of their false negatives were actually weakly positive test lines.

The study had two main limitations: Firstly the sample size was small; thus generalizations about the study results need to be interpreted with caution. However, the sample was carefully chosen using multi-stage stratified random sampling, and is thus representative of RDT end-users in the study area. Moreover the study does raise similar concerns about weak positive results as other studies (59, 62), and thus the findings may be relevant to similar settings in South Africa and in Africa where RDTs are being used. It was not possible to increase the sample size for logistical and operational reasons - clinics were in very remote parts of the Limpopo Province and could only be accessed by dirt road; the student was asked not to disturb service delivery in the clinics and had to wait for up to 3 hours to interview one of the end-users - and because of financial constraints.

Secondly the use of photographs of a pre-prepared battery of tests as a gold standard to determine sensitivity and specificity of end-user interpretation may have been a limitation. Photographs may not be the best gold standard as they may have captured the test lines differently to what end-users were used to. Photographs of weak positive results may have appeared lighter than what they truly where – and this could have distorted end-user's interpretation of the results, and in particular, of weak positive results. The actual tests (instead of photographs) could not be used to assess end-user performance as the signals on these test deteriorate over time, making it difficult to use acual tests to assess end-user interpretation. This is especially so for weak negative results. Thus photographs of a battery of pre-prepared tests were used to assess end-user MRDT interpretation.

5.7 Conclusion

The end-user proficiency (performance and interpretation of results) for MRDT in the Vhembe District of the Limpopo requires improvement. This study has indicated that those end-users who were trained were more proficient at using MRDTs than those who were not trained. End-user training should therefore be one of the key factors for health authorities to consider when addressing the challenges of end-user MRDT proficiency in the Vhembe District.

The key gap identified was adherence of end-users to the key steps in the MRDT test procedures and mis-interpretation of weak positive and ambiguous test results.

6.0 Chapter 6: An assessment of the feasibility of PCWs for MRDT Quality control

6.1 Introduction

Quality control of MRDT is important for optimising accuracy of MRDTs and ultimately improving malaria diagnosis.(9, 22, 67) An appropriate quality control system for the South African setting would be to use positive control wells (PCWs). (66, 68)The currently available PCWs in South Africa is manufactured by National Bio-products Institute, did undergo laboratory evaluations for signal strength and stability, with favourable outcomes (personal communication with manufacturer- no published data was available as PCWs were still being finalized at the laboratory level at the time of this study). However it was not tested to determine its reliability under field conditions. Therefore an assessment of the PCW to determine its applicability for routine quality controlling of MRDTs in the field was carried out.

6.2 Objectives

(1) To determine the reliability of PCWs in field conditions.

(2) To compare the reliability of HRPII negative blood versus citrate buffer for diluting PCWs

6.3 Study methods

The study was conducted on 18 randomly chosen clinics and 5 corresponding referral hospital laboratories in the Vhembe district, (see Appendix 17). A centralized approach and a remote testing approach was used to determine overall reliability of PCWs in field conditions. In both approaches MRDTs that were used were stored at the clinic level thus only the PCWs were stored differently. In the remote approach PCWs and MRDTs were stored and tested at the selected clinics. In the centralised approach PCWs were stored at a referral hospital laboratory and tested in the

hospital laboratory using selected MRDTs obtained from the clinic. The brand of MRDTs tested where those that existed at the clinics at the time of the study.

6.3.1 Approaches to quality control

6.3.1.1 Remote testing approach to quality control

Two PCWs were transported to each of the 18 study clinics and stored at room temperature for a period of approximately 1 month. MRDTs were stored at clinics as per the clinic protocol. After a period of one month, 4 MRDTs were randomly selected from the stored batch of the tests and tested in the laboratory, according to the study protocol (see below).

6.3.1.2 Centralised approach to quality control

2 PCWs each were transported and stored in 5 selected laboratories (see Appendix 17) for a period of one month. After one month four unopened and unused MRDTs were randomly selected from the oldest batch of the MRDTs stored at each of the 18 study clinics and transported to the central referral hospital laboratory and tested in the laboratory, according to the study protocol (see below). MRDTs were transported in cooler boxes, according to the manufacturer's recommendation.

6.3.2 Study protocol for MRDT testing of PCWs

Under field conditions at clinic and hospital levels the following was performed:

- The first MRDT was tested using a known HRPII positive blood sample to establish if the batch was working,
- The second MRDT was tested using the citrate buffer only (which was negative for malaria) to establish if the batch was working,
- the third MRDT was tested with the stored PCW diluted with known HRP II negative blood sample (stored in an EDTA tube) and
- the fourth MRDT was tested with the stored PCW diluted with citrate buffer (provided by the manufacturer).

All results were recorded on a data recording sheet (See Appendix 18).

If the first or second MRDT gave a false positive or negative result, respectively then remaining 2 MRDTs were not tested, further, as it was assumed that the batch was 'not working'. The storage condition and duration of storage of each MRDT in the study clinics were observed and documented.

The strength of the MRDT result was assessed using a colour intensity chart (see Appendix 19) adapted from Lon et al. and other reports: (9, 68)

- no colour (negative) =0;
- barely visible = 0.5;
- weak positive =1 and
- strong positive =3.

6.3.3 Data analysis

The main aim of the study was to determine whether PCWs could be considered for a quality control programme for routine sensitivity monitoring of HRP II antigen-detecting MRDTs in the Limpopo Province. Eighteen clinics were randomly selected and 5 hospital laboratories were purposively selected so that reliability under different conditions could be assessed. Results are presented for the overall sample (PCWs in clinic and hospital laboratories). The reliability of PCWs was assessed using the signal strength. Factors that could affect the reliability of PCWs such as type of MRDT, distance of clinic from the referral hospital laboratory, storage temperature and type of diluent (blood or citrate buffer) used to dilute the PCWs are described and were taken into account in this analysis. Factors that could affect the outcomes of the PCWs were selected from WHO manuals and published literature and communication with the manufacturer of the PCW.(9, 68)

6.4 Ethics

Although the student performed all tests, informed consent for this aspect of the study was obtained from chief technologists in each laboratory, from the head of

each selected laboratory and from senior nurses from health facilities respectively, see Appendix 20 and 21 for study information sheets and informed consent forms.

6.5 Results

6.5.1 Description of key variables and outcomes.

In the 23 sites health facilities (18) and hospital laboratories visited (5), 7 had ICT diagnostic testing kits and 16 had Makromed kits. The median distance between the clinics and nearest the hospital laboratory was 42.5 km (IQR=19). The median number of days for which PCWs were stored at the health facilities was 33 (IQR=2), with the median temperatures of the facilities being 21^0C (IQR=4). One hundred percent (23/23) of the known Pf positive blood samples were positive on the MRDTs (ICT and MAKROMED), thus indicating that the test kits were working and the batches were validated as being effective. A negative control (buffer run alone on the test strip) tested negative for each batch the MRDTs that were used. Sixty percent of the PCWs (clinic and lab) diluted with the HRP II Negative blood was positive; whilst approximately 9% of PCWs (clinic and lab) diluted with citrate buffer was positive. See Table 17 and 18 for detailed findings.

Variable	Description	
	N(%)	Total
Type of tests used	ICT 7 (30.43) Makromed 16 (69.57)	23
Testing approach	Clinics 18 (78.25) Hospitals 5 (21.74)	23
median distances from hospital laboratories	42.5 (19*); range 8-87km	18
Median storage days of PCWs at clinics centres	33 (2*); range 17-38 days	23
Median temperatures	21 (4*); range 20-33^{0}C	23

Footnote: *=IQR

Table 17: Description of key QC variables

Outcome Variables	N (%)	N
Test results with positive blood sample	Pos 23 (100)	23
Test results with citrate buffer	Neg 23 (100)	23
PCW results diluted with HRP II negative blood	Neg 9 (39.1) Pos 14 (60)	23
PCW results diluted with Citrate buffer	Neg 21 (91.3) Pos 2 (8.7)	23

Table 18: Positive control well outcomes

6.5.2 Comparing test outcomes with different diluents

Diluting the PCWs with HRP II negative blood, proved better than diluting with citrate buffer - the citrate buffer diluent yielded more false negatives than the HRP II negative blood diluent. This difference was consistent for all the factors affecting the outcomes of PCWs (Table 19). The following are key findings:

- Using the Makromed test kit (n=16) all 16 PCWs diluted with citrate buffer tested negative whilst 4/16 (25%) diluted with MRDT negative blood tested negative.
- When tests on PCWs stored for up to 33 days were performed (n=15) all 15 PCWs diluted with citrate buffer were negative compared to 4/15 (27%) diluted with HRPII negative blood.
- When tests on PCWs stored at >26^{0}C were performed (n=5) all 5 PCWs diluted with citrate buffer were negative compared to 3/5 (60%) diluted with HRP II negative blood.
- Lab stored PCW's (n=5) diluted with citrate buffer HRPII yielded 20% (1/5) negative results whilst PCWs diluted with HRP II negative blood gave no negative results.
- Of PCWs stored at clinics (n=18) 50% (9/18) diluted with citrate buffer were falsely negative whilst 44% (8/18) diluted with HRPII negative blood, were falsely negative.

Factors Affecting the quality of PCWs		Malaria Positive blood sample (test positive/ total tested)	PCW diluted with HRP II negative blood (false negative/total tested)	PCW diluted with citrate buffer test (false negative/total tested)
Brand of MRDT	ICT	7/7 (100%)	5/7 (71.4%)	5/7(71.4%)
	Makromed	16/16 (100%)	4/16 (25%)	16 (100%)
Type of facility	Hospital Lab	5/5 (100%)	0	1/5 (20%)
	Clinic	18/18 (100%)	8/18 (44.4%)	9/18 (50%)
Number of days stored in the clinic/ Hospital	17-30	3/3 (100%)	1/3 (33.3%)	3/3 (100%)
	31-33	12/12 (100%)	3/12 (25%)	12/12 (100%)
	34-38	8/8 (100%)	4/8 (50.0%)	6//8 (75%)
Median storage temperature	20-25	18/18 (100%)'	6/18 (33.3)	16/18 (89.2%)
	26-30	4/4 (100%)	2/4 (50%)	4 (100%)
	31-33	1/1 (100%)	1 (100%)	1(100%)

Table 19: Comparison of test outcomes of PCWs using different diluents

6.6 Discussion

All the MRDTs tested were in good working condition as all 23 MRDTs collected from the clinics were strongly positive (tested against the positive colour chart) when using the known positive HRP II stored blood . Similarly the buffer when tested alone (negative control) on the MRDT test strips was consistently negative when tested each time the PCWs were being evaluated.

PCW performed better when diluted with HRP II negative blood than diluted with citrate buffer. The reasons for this could be that the MRDTs were designed to test blood samples (personal communication Dr Martin Bubb, National Bio-products), hence the poor overall performance of the citrate buffer. The WHO states that citrate buffer will ideally last longer than blood, as a diluent.(66) However, in this study blood was a better diluent for the PCW. Logistically it would not be that difficult for health facilities to store HRP II negative blood as they are all equipped with refrigerators and malaria control teams visit clinics once per week hence the HRP II negative blood samples could be replenished. However the challenge still remains for the development of a suitable synthetic buffer for diluting PCWs as this will be an ideal diluent- as the shelf life to the diluent is longer than that of whole blood.

6.6.1 PCW diluted with HRP II negative blood

The MRDT kit type was not initially chosen as one of the variables when the study was designed, however during the course of this study it was noted that some clinics had retained the Makromed testing kits as they were in surplus and, hence were still using them. This provided an opportunity to compare the PCW results and signal strengths on the Makromed Kit with the ICT diagnostic test kits. One of the possible reasons for the Makromed kit performing better than the ICT kit when testing the PCWs (after diluted with negative HRPII blood) could be due to the antigenic variation. Lee et al. observed a significant difference in the reactivity of the same monoclonal antibodies (MAB) to different *P. falciparum* isolates. (99) When the target epitopes of three MABs were determined and mapped onto the peptide

sequences of the field isolates, significant variability in the frequency of these epitopes was observed. (110) Antigenic variation would therefore need to be addressed when developing PCW's, such as that used in this study, so that at least WHO approved MRDTs can be quality controlled using this method .This should include ICT Pf and the Makromed testing kits currently used in South Africa. MRDT trials that have been reviewed on acceptable MRDTs are available on WHO MRDT website hence PCW manufactures should consider selecting from this list. (24)
This study showed variability on the signal strength when PCWs were stored for more than 30 days. In the study setting it is possible for PCWs to be replenished every 2 weeks, hence the current PCW can be used, however, health facility managers, would need to ensure that PCW expiry dates are regularly monitored, similar to that of the MRDT kits.

6.6.2 PCW diluted with citrate buffer

The two PCWs diluted with citrate buffer that were positive after storage of 34-38 days, could be due to non specific reactions (personal communication with PCW manufacturer), especially due to the fact that all those stored at ≤ 33 days were negative. Citrate buffer quality for contamination over 30 days would need to be tested if it is to be further considered for use as a diluent for PCWs.

6.7 Conclusions

This evaluation of PCWs shows that PCWs can be used as a tool for monitoring MRDT quality at the field level (clinic level) and suggests that HRP II negative whole blood is a better diluent than citrate buffer. PCWs will need to be further developed before routine implementation at clinic level, operational issues such as training of the end-user (clinic staff) and transport logistics and storage of PCWs will also need to be addressed. The use of citrate buffer needs to be further explored as a diluent; blood is not ideal due to challenges with storage and transport logistics.

7 Chapter 7: Discussion and Conclusions

The early and accurate diagnosis of malaria is important to ensuring prompt treatment for malaria.(111) Several methods exist to diagnose malaria and each method has useful applications in specific settings.(9, 67) Whilst the gold standard for malaria diagnosis remains malaria microscopy its applicability to the field/ primary health care settings is questionable. (12, 57, 112, 113) This is due to skilled microscopists and reliable equipment not being available and microscopy taking time to conduct.

MRDTs are the second best malaria diagnostic tool that can be deployed in large-scale in primary health care settings.(12, 22) Whilst MRDTs are an efficient tool for diagnosis of malaria, there are several factors that can affect its accuracy.(9, 67) As treatment at the primary health care levels in South Africa is based on definitive diagnosis using MRDTs, ensuring quality and accuracy of MRDTs at field level becomes extremely important.(8) In this study the key factors, in the opinion of the end-users that could affect the quality and usage of MRDTs was determined. Subsequently the field accuracy of MRDTs used in South Africa and the proficiency of end-users were evaluated. Finally the feasibility of setting up a quality control system was assessed.

In this chapter the limitations of the study, the key observations and their public health implications are discussed.

7.1 Temperature monitoring of MRDTs

The lack of cooling facilities to store MRDTs was noted as a challenge. Eighty five percent of clinics in malaria areas had air-conditioning equipment and were monitoring temperatures. However both MRDTs and PCWs that were stored at more that 25^0C showed a tendency for weaker to no signals. Chiodini et al. and Jorgensen et al; have shown in their heat stability studies of HRP II based MRDTs that the performance of malaria RDTs can be adversely affected at the temperatures

to which they will be exposed when transported to, and used in the field.(74, 75) These studies and others suggest that random checking of temperatures in pharmaceutical storage rooms of clinics would be an important step in MRDT quality assurance.(54) Coupled with this, MRDTs should be considered for batch testing prior to being received by the pharmaceutical depots at the districts and subsequent to field exposure and field conditions, by reference laboratories. The standard WHO guideline should be considered to undertake batch testing.(54)

7.2 Sensitivity of ICT Pf test

The overall sensitivity of the ICT Pf test is above the threshold of 95% that is recommended by the WHO.(9) However the sensitivity at 100 parasites/ µl of blood could not be ascertained in this study as all the slides did not have parasitaemia counts conducted on them due to the poor quality of slides. Among those slides in which parasite counting was possible none had parasite levels of 100 parasites/ µl of blood and below. Late presentation of malaria patients to the health facilities which often occurs in the district, could be one possible explanation for this. Laboratory parasite dilution studies did reflect that the ICT Pf test was 100% sensitive at 70 parasites µl of blood.(87) Whilst this evidence maybe sufficient to support the usefulness of the ICT Pf test, health authorities should consider a larger study with more clinics and increased sample size to test the accuracy of ICT Pf to diagnose malaria with a parasite density of ≤ 100 parasites/ µl of blood.

7.3 False negative MRDTs

False negative malaria diagnosis was low in this study (1/405 patients tested), however applying this level of false negative rate to 51 712 febrile patients per year reported in the study clinics suggests that there could be 128 false negative false negative diagnoses per year.

Although the number of malaria related deaths in the false negative patient's in the study was zero, the true mortality from the study population could not be ascertained

as all the patients did not return for follow-up. Thus, the possible deaths among the assumed "128 false negative results" cannot be estimated. The reasons for false negative diagnosis of malaria therefore become critical and should be determined, in the study population. The end-user interpretation of results and the quality of microscopy are factors needing improvement. In particular the operational deficiency among the end-users such as usage of inadequate amounts of buffer and incorrect reading times to read the MRDTs and poor interpretation of weak-positive MRDTs results should be addressed.

7.4 False positive ICT results

Whilst false positive diagnosis of malaria can result in over-treatment and possible drug resistance, altering the physical test mechanism by the manufacturer may not be appropriate.(16, 22) Several authors have found that in a highly sensitive test, specificity tends to decrease. In the case of the ICT Pf test the specificity of 96% was above WHO acceptable criteria.(9) Again the quality of the gold standard, viz microscopy needs to be improved as the weak positives could be missed even by very skilled microscopists.(9, 22)

7.5 Quality of microscopy

The quality of microscopy by field microscopists showed deficiencies, despite the quality of microscopy slides not being optimal. The discordance between field microscopists of approximately 5% is cause for concern, especially when high level parasitaemias were missed. This is contrary to what other studies have found, where low level parasitaemias were missed by microscopy.(85, 86) It is therefore imperative for health authorities of the Vhembe district to conduct a study on the status of malaria microscopy at the field level, especially when microscopy is sometimes used to confirm diagnosis and to monitor patient treatment responses. (8)

7.6 Predictors of malaria

As the diagnosis of malaria in South Africa especially at the primary health care level is made mainly by nurses using MRDTs, the challenge lies in situations where patients are ICT negative but there is a high malaria clinical index of suspicion. (8)

The situation becomes more complicated when health facilities are a considerable distance away from laboratories. The guidelines for the treatment of malaria and the Integrated Management of Childhood Illnesses chart booklet state that in such cases the treatment for malaria should be administered and the patient should be referred to the hospital.(82) Ndyomungyenyi et al. has noted in their study Uganda that fever may not be the main predictor of malaria.(21) Their study found that headache (84.4% n=633) followed by joint pain (78.1%; n=586) were stronger predictors for malaria and then followed by fever (72, 2%; n=579) in their study population. Although headache had lower sensitivity (52.23%) than fever (64.58%) for predicting malaria), sweating (81.25% n=394) and chills (72.83%; n=394) showed higher sensitivities. This emphasizes the point that clinical predictors may differ from one setting to another and are not very useful for making diagnostic and treatment decisions. The accuracy component of the study showed that patients' reporting a history of sweating is a significant predictor of malaria. Therefore, the validity of the perceived patient symptoms of malaria in the Vhembe district needs to be robustly evaluated and the guidelines for testing patients for malaria should be revised accordingly.

7.7End-User Performance

Training of end-users was observed as a challenge. Interestingly, the nurses who had received training were 3.75 times more likely to perform the MRDTs and 2.9 times more likely to interpret the results better than those who did not receive training. Training to ensure that malaria RDTs are optimally used and interpreted has been advocated by several studies and reports.(9, 46, 59, 64, 67, 114) Health authorities in the Vhembe district should therefore make training a key activity in their operational planning. Training guides are available to guide health managers on how to use and interpret MRDTs. Interpretation of weak/faint positive MRDTs needs to be highlighted in the training.(9)

7.8 Quality control for MRDTs

Positive control wells are a novel method for addressing the quality control of MRDTs after exposure to field conditions.(9) The PCW evaluated for the quality control component of this study has shown some promise for use at the clinic level, similar to the findings of Lon et al.(68) However the stability of the PCW- after storage at field level without significant loss of antigen activity is a crucial step before this technology can be considered for wider field implementation. In this study the field stability of the antigen with the HRP II negative blood diluted PCW (better of the 2 diluents) showed variability in signal strength; hence this would be the key issue that needs to be addressed before considering field application. The practicalities for implementing PCWs at the field level will also need to be assessed, issues of storage environment and usage by end-users will be important.

7.9 Conclusions

The exploratory study revealed that MRDT storage, quality control, end-user training and use of MRDT results for clinical decision making in primary health care facilities in study setting need to be improved. Furthermore detailed studies on MRDT accuracy; end-user performance and developing quality control for MRDTs are needed. For this reason subsequent studies on evaluation of the field accuracy of ICT Pf test and end-user performance in conducting and interpreting MRDT results and to determine whether positive controls could be used to routinely monitor MRDT quality in the field, were conducted.

The overall diagnostic accuracy of ICT Pf MRDT, judged by the sensitivity and specificity of the test, is of acceptable levels as measured against the WHO standards. However the sensitivity of the ICT Pf at the field level, at 100 parasites/µl of blood was not determined in this study and would need to be ascertained in a larger study with a representative sample. These findings were communicated to the Department of Health in South Africa and malaria managers are confident to continue with using the ICT Pf diagnostic test. The protocol for this study was also

shared with the Malaria Control Programme in the Limpopo Province - which has undertaken to regularly monitor the field accuracy of MRDTs; determine the lower parasite density detection limits and compare it with the WHO acceptable limits (≤100 parasites / µl of blood).

The MRDT findings using the ICTpf diagnostic test (from Global diagnostics in South Africa), shows a high level of accuracy - sensitivity and specificity and good positive and negative predictive values. Countries with similar malaria transmission patterns and in same geographical locations such as South Africa (e.g. Botswana, Namibia, Swaziland, Zimbabwe) can consider using the ICTPf test for diagnosis. Due to the relatively low malaria transmission in parts of these countries there maybe insufficient positive samples to evaluate the ICTPf test, hence the finding from this study becomes important when they considering adoption of this test for diagnosis of malaria in their countries.

The effect of the environment on the quality ICT Pf MRDT requires further study as heat instability during transport and storage of MRDTs was not determined in this study.

Inter-observer variability among microscopists was reliable. However 5% disagreements especially on slides with a parasite density >100 parasites / µl of blood is a concern. Thus microscopists would require retraining and regular quality assurance assessments.

Training of end-users on operational aspects and interpretation of results should be considered as a priority by district health managers in the Vhembe districts. The use of standardized guidelines needs to be considered as this will ensure quality results by all levels of RDT end-users, both experienced and new staff members.

This study found that after controlling for other reported symptoms of malaria, patients presenting with sweating were 5 times more likely to be slide positive for malaria than those whom did not have sweating. As fever is the main clinical criteria

for testing patients for malaria in the IMCI guidelines for South Africa and the National Malaria Treatment Guidelines, malaria could be under-diagnosed in patients presenting with other reported symptoms and no fever. A broader study using all the signs and symptoms of malaria therefore needs to be considered for determining predictors of malaria especially for situations where there is a high clinical index of suspicion of malaria, laboratories are not readily accessible and RDT results are negative.

PCWs are a novel way of achieving field based quality control. Quality control of MRDTs after exposure to the field is crucial to ensuring accurate diagnosis. This study shows that PCWs may be used for quality control of MRDTs and has made a start to addressing quality control of MRDTs at the clinic level but it would need to be taken further to determine its operational and technical feasibility for wide scale field application. The malaria control programme of the Limpopo Province will need an interim quality control system to monitor their MRDTs post-field exposure, whilst PCWs are being optimized. Given the current options available the prepared quality control sample using wild type antigens as suggested by WHO should be considered for interim use. The quality control sample can be prepared at a central reference laboratory (at the National Institute for Communicable Diseases-that has the technology and skills) and transported and stored at district hospital laboratories in the Limpopo. MRDTs could then be randomly selected from the clinics, transported and assessed at district hospital laboratories, with support from the district malaria control programme officers.

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9.0 APPENDICES

9.1 Appendix 1: Clinics and Health Centres visited for the Exploratory Study

DISTRICT	MUNICIPALITY	FACILITYNAME	2002	2003	2004	Average
BOHLABELA	BUSHBUCKRIDGE	Belfast Clinic	8	16	15	13
BOHLABELA	BUSHBUCKRIDGE	Gottenburg Clinic	15	7	20	14
BOHLABELA	BUSHBUCKRIDGE	Matikwana Hospital	127	61	180	122.67
BOHLABELA	BUSHBUCKRIDGE	Thulamahashe Health Centre	29	138	40	69
BOHLABELA	MARULENG	Agincourt Health Centre	2	16	14	10.67
MOPANI	BA-PHALABORWA	Homulani Clinic	43	24	51	39.33
MOPANI	BA-PHALABORWA	Lulekani Health Centre	126	125	61	104
MOPANI	BA-PHALABORWA	Namakgale Clinic	13	13	20	15.33
MOPANI	BA-PHALABORWA	Phalaborwa Hospital	107	106	131	114.66
MOPANI	GREATER GIYANI	Dzumeri Health Centre	56	44	18	39.33
MOPANI	GREATER GIYANI	Giyani Health Centre	12	54	80	48.66
MOPANI	GREATER GIYANI	Kremetart Clinic	0	11	46	19
MOPANI	GREATER GIYANI	Mavambe Clinic	11	16	3	10
MOPANI	GREATER GIYANI	Tomo Clinic	176	249	114	179.66
MOPANI	GREATER GIYANI	Nkhensani Hospital	72	396	411	293
VHEMBE	MUSINA	Madimwo/Madimbo Clinic	146	202	121	156.33
VHEMBE	MUSINA	Messina Hospital	336	307	315	319.33
VHEMBE	MUSINA	Nancefield Health Centre	18	21	42	27
VHEMBE	MUTALE	Tshipise Clinic	87	70	96	84.33
VHEMBE	THULAMELA	Mhinga Clinic	99	129	32	86.67
VHEMBE	THULAMELA	Mphambo Health Centre	21	40	16	25.67
VHEMBE	THULAMELA	Shikundu Clinic	18	16	6	13.33
VHEMBE	THULAMELA	Tshilidzini Hospital	229	450	181	286.66

9.2 Appendix 2: Interview Schedules for exploratory study to determine which factors affect the accuracy of MRDTs

Key informants

Group1: Operational and Pharmaceutical service managers at district and Provincial level (n=18).

Group 2: Nursing sisters working in clinics and health care centers (n=17).

Group 3: Scientists in Research Institutes (n=2).

Group 1.

For Key Informants:

1. What processors were used to decide on policy for using RDTs
2. What are the criteria used for purchasing Rat's for malaria diagnosis in your province/district/locality?
3. Could you list the manufactures of the RDTs that you purchased from, over the last one-year period and how long each type of RDT was used for?
4. Could you list the suppliers of the RDTs that you ordered over the last one year period ? How long has these supplies been around for?
5. What is the range of prices of MRDT's that you ordered last year?
6. Could you describe the procedures for distribution of MRDT's to clinics, health centers and hospitals?
7. Did you have adequate supplies of RDTs throughout the year?
8. Do you have a contingency plan to ensure the rapid distribution of RDTs if they are needed during an outbreak? Describe this plan
9. Could you describe the procedures for storing RDTs? (at the central depot and at a clinic?)
10. How are the expiry dates of RDTs monitored?
11. Do you follow any guidelines for RDT use? If yes, which guidelines do

you follow? Have these guidelines been adopted for use in your district?

12. Do you test the quality of RDTs? If yes, what procedures are used for testing RDT quality?

13. Where there any reports of failures of RDTs ?

14. Do you do any further tests to confirm failure of RDTs?

15. Is there an operating procedure for testing quality of RDTs?

16. Are any confirmatory tests used? If yes, what criteria are used to determine when they should be used? What confirmatory tests are used ?

17. In your opinion, does the staff at the health centers and clinics have adequate skills for using RDTs?

18. Has there ever been a formal assessment of staff skills on RDT procurement and use? If yes, where and when was this done, how was it done and what were the results

19. In your opinion do staff strictly follow the guidelines for interpreting RDTs results?

20. In your experience, what do staff do if the RDT results are negative?

21. How often is training provided for staff to perform RDTs?. Which staff are trained? Are trained staffs expected to train other staff at their facilities or are all staff trained at a central point?

22. How often is staff followed up? What is done on follow-up? What have been the findings during such follow-up ?

23. What do you feel are the strengths of using RDTs?

24. What are the challenges with using RDTs?

Group 2

End-users:

1. Where are malaria RDTs stored?
2. How are malaria RDT stocks monitored?
3. Do you check the expiry dates of RDTs in stock? If yes how often?
4. What is the procedure for ordering malaria RDTs?
5. How do you estimate the quantity of RDTs you need for each ordering cycle?
6. Do you test the quality of RDTs? If yes how often do you test the quality?
7. How is RDT quality (accuracy – whether they work) monitored at the clinic/ health care center?
8. How confident are you with the results of the MRDT's you use?
9. Have you given antimalarials to a patient whose RDT test was negative? If yes. How often patients with negative MRDT's receive treatment?
10. Do you always give an antimalarial to a patient whose RDT test is positive? If no; how often patients with positive MRDT's did not receive treatment?
11. In your opinion, are RDTs easy to use? If, yes, on a scale of 1-5 how easy are RDTs to use - 5 is very difficult and 1 is very easy
12. Are you confident in interpreting the RDT results?
13. When did you receive training (the last one if more than one) in testing for malaria using RDT?
14. Is the RDT used in your clinic/HC the same on which you were trained?
15. What further support you receive for using MRDT's? If yes who gives it and how often?
16. What do you do when you run out of stock of RDTs?
17. Have you ever run out of stock of RDTs? When? For how long did this last? What did you do?
18. Do you have a plan for ordering RDTs in an emergency? What is this plan?

19. Do you follow any guidelines for RDT use? If yes. which guidelines do you follow?

20. What can you describe as positive aspects of using RDTs?

21. What can you describe are the negative aspects of using RDTs?

Group 3

Research Institutions:

1 What is your experience with Quality Control of malaria RDTs?

2 What is the process of testing RDTs after they are purchased from the suppliers/manufactures?

3 Are RDTs from different manufacturers regularly tested and compared?

4 Are RDTs from different manufacturers and suppliers of similar quality?

5 What is the level of skills for RDTs testing at the laboratory and the field level?

6 Is there existing capacity at the field level and Laboratory level adequate?, for testing RDT quality. If no what training would be required.

7 What confirmatory tests are performed to validate the MRDT's results?

8 In your opinion, does the appropriate Infrastructure and technology exists for RDT testing at laboratory level? If no, what changes are needed to ensure that the appropriate infrastructure and technology exists?

9 What do you perceive as a strengths/opportunity for quality control of RDTs at the laboratory and field level?

10 What would you say are some of the challenges for quality control of RDTs at the laboratory and the field level?

9.3 Appendix 3: Participant information sheet - Exploratory study

Information Sheets & Consent forms

PARTICIPANT INFORMATION SHEET

TITLE OF RESEARCH PROJECT:

Factors that affect the quality of Malaria Rapid Diagnostic tests, in the Limpopo Province of South Africa

Prinicipal Investigator:
Devanand Moonasar: National Department of Health South Africa and London School of Hygiene & Tropical Medicine, UK

Contact Information:
Tel: +27 (0) 12 3120102
Fax: +27(0) 12 3123113
e-mail: moonad@health.gov.za/patrick.moonasar@lshtm.ac.uk

WHY ARE WE DOING THIS STUDY?

Malaria is a disease that can be treated and cured. Early diagnosis and treatment is important for preventing severe or complicated malaria and death. Since Rapid Malaria Tests are being used for Malaria diagnosis in your area, it is important for these tests to be accurate and easy to use. We are doing this study to determine what are the factors that affect the accuracy of Rapid diagnostic tests and to determine how well the test performs under field conditions. Before starting a large study we would like to find out what are your views on Rapid Diagnostic Tests to detect where problems may lie. This information given by you will help us to develop a larger study to address the questions on the use of Rapid diagnostic tests. The interview will last approximately one hour. We are planning to interview about 28 people who are involved with Rapid Diagnostic tests for diagnosis and management of malaria.

DO I HAVE TO TAKE PART?

It is entirely up to you to decide whether or not to take part in this study. If you decide not to take part, this will not affect you in any way. If you do decide to take part you will asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

IF I TAKE PART IN THIS STUDY, WHAT WILL HAPPEN?

After you have had a chance to ask questions, and have signed the informed consent form, we will ask you some questions for approximately 1 hour. If you give

your permission, we will tape record the interview. If you do not give permission, we will take notes on paper. We will ask you about issues that help you and issues about Rapid Diagnostic Tests, e.g. how you use them, what problems you find with them. You are free to stop the interview at any time or to not answer any questions that you feel uncomfortable with.

WHAT ARE THE RISKS AND BENEFITS OF TAKING PART IN THIS STUDY?
There are no risks in taking part in this study, but we would ask for about 1 hour of your time today. This study may not benefit you right now, but the results of the study will help us to know what difficulties you have with Rapid Diagnostic tests and how we might improve this. It will also help sisters in this clinic and health centres to use Rapid Diagnostic test with ease and managers to purchase the best tests for your setting.

WHAT ARE THE COSTS TO ME?
There is no cost to you for participating in the study.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?
All information collected, as part of this study will be kept securely and confidentially. Mr Devanand Moonasar the lead researcher for this study will be responsible for this. Your name will not be recorded in this interview. You will not be personally identified in any report about this study. All information will be kept in a locked and secured cabinet.

WHAT IF I HAVE MORE QUESTIONS I WISH TO ASK ABOUT THIS STUDY?
If you have any questions about this study, please ask us now. We will also give you a copy of this information sheet, which explains the study to take away with you. If you have questions later you can telephone Mr. Devanand Moonasar on 012 312 0102. The committees giving ethical approval for this study are the Research Ethics Committees of the London School of Hygiene and Tropical Medicine, UK and the Medical University of South Africa.

CONSENT FORM

Title of project: Factors that affect the quality of Malaria Rapid Diagnostic tests, in the Limpopo Province of South Africa

Investigator:
Devanand Moonasar: National Department of Health South Africa and London School of Hygiene & Tropical Medicine, UK

CONSENT TO PARTICIPATE IN THE RESEARCH STUDY

- I have read the information sheet about this study and I understand what will be required of me and what will happen to me if I take part in the study.

- I understand that I may withdraw from this study at any time without giving a reason and without affecting my normal care and management.

- I agree to take part in the study **YES / NO** (answer to be circled)

Participant's signature :_____

Date: _____

Participant's name: _____

9.4 Apppendix 4: Selected Clinics and sample size calculation for Accuracy study (Component 1)

District	Municipality Name	Clinic Name	Malaria Cases				
			2002	2003	2004	2005	Average
VHEMBE	MUSINA	Madimwo/Madimbo Clinic	146	202	121	104	156.3
VHEMBE	MUTALE	Mulala clinic	157	239	168	164	188

The number of malaria patients that were needed to be included in the study to determine sensitivity and specificity was based on the 6 month average malaria clinic prevalence amongst patients with suspected malaria for both clinics – which was 4.95%; an additional 10% was added for loss to follow-up, bringing the final sample size to 406, see box 4.

Sample size

To determine sensitivity and specificity

$$n \geq \frac{(1.96)^2 p(1-p)}{X^2}$$

$$= \frac{(1.96)^2 (0.95)(1-0.95)}{(0.1)^2}$$

where:
p= suspected MRDT sensitivity = 95%
X = 10% or 0.1 (confidence interval within which the test sensitivity will be measured).

= **18.25 MRDT-positive patients**

I needed to recruit 18.25 MRDT-positive patients to determine sensitivity to within 10%.
Number of patients with suspected malaria over a 6-month period = 4000 (2 clinics: Mulala and Madimbo)
The number of malaria positive cases in these 2 clinics over the same six months was 198
Thus the prevalence of malaria amongst patients presenting with symptoms suggestive of malaria was 4.95 % (198 /4000* 100) over a 6-month period.
This implies that [(100/4.95)*18.25] = **369 patients** with symptoms of malaria need to be screened to get 18.25 MRDT-positive cases per site
Thus the total sample size for two clinics was calculated as 369.
Allowing for a 10% loss to follow-up I increased the total sample size to **405.**
Reference for malaria prevelance. (115)

Sample size calculation for accuracy study

9.5 Appendix 5: Microscopy recording sheets

PAGE 1. Evaluating the Performance and Usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo, South Africa		
Blood Smear Results: MADIMBO Clinic		
Study ID Number	Thick Smear results	Comments
MAD 001		
MAD 002		
MAD 003		
MAD 004		
MAD 005		
MAD 006		
MAD 007		
MAD 008		
MAD 009		
MAD 010		
MAD 011		
MAD 012		
MAD 013		
MAD 014		
MAD 015		
MAD 016		
MAD 017		
MAD 018		
MAD 019		
MAD 020		
MAD 021		
MAD 022		
MAD 023		
MAD 024		
MAD 025		
MAD 026		
MAD 027		
MAD 028		
MAD 029		
MAD 030		
MAD 031		
MAD 032		
MAD 033		
MAD 034		
MAD 035		
MAD 036		
MAD 037		
MAD 038		
MAD 039		
MAD 040.....etc.		

Evaluating the Performance and Usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo, South Africa		
Blood Smear Results: Mulala Clinic		
Study ID Number	Thick Smear results	Comments
MUL 001		
MUL002		
MUL 003		
MUL 004		
MUL 005		
MUL 006		
MUL 007		
MUL 008		
MUL 009		
MUL 010		
MUL 011		
MUL 012		
MUL 013		
MUL 014		
MUL 015		
MUL 016		
MUL 017		
MUL 018		
MUL 019		
MUL 020		
MUL 021		
MUL 022		
MUL 023		
MUL 024		
MUL 025		
MUL 026		
MUL 027		
MUL 028		
MUL 029		
MUL 030		
MUL 031		
MUL 032		
MUL 033		
MUL 034		
MUL 035		
MUL 036		
MUL 037		
MUL 038		
MUL 039		
MUL 040		
MUL 041		
MUL 042		

9.6 Appendix 6: Standard operating procedure

Roles and Responsibilities of nurses in the DoH malaria study

Introduction

This study aims to determine the management of patients with fever, headache and / or chills in the Limpopo Province. We hope to determine the accuracy of malaria rapid test kits and how patients with fever are managed and what their outcomes are, with a view to improving health service delivery and patient care in the Limpopo province and in South Africa. Your assistance in this study is thus very important for South Africa.

You will need to undertake 3 main tasks for the successful completion of this study:

- Identifying eligible patients
- Obtaining informed consent
- Obtaining and documenting data

A. IDENTIFYING PATIENTS

When to recruit

1. After you have managed / diagnosed / treated the patient for their presenting illness, focus on recruiting them for this malaria study.

Who to recruit

2. Do not recruit very sick patients, patients with danger signs or patients with coma/ unconsciousness / pneumonia / diarrhea or children with severe IMCI classifications. So - if your patient has any danger sign or a severe IMCI classification or pneumonia or diarrhea then he/she should not be recruited for this study. You should give these patients the routine care that is provided in your clinic.
3. Discuss the DoH malaria study with each patient that has the following signs / symptoms: fever, chills, sweating and headache.

B. OBTAINING INFORMED CONSENT

What should you tell patients

4. Explain to each patient with these signs/symptoms that a study is being conducted to determine how patients with fever or headache or chills or sweating) symptoms are managed and what their outcome will be.
5. Explain that their participation in the study will assist to improve the care of people in the Limpopo. The study will also help <u>them</u> by following them up and making sure that they receive optimal treatment. It will also help other

patients in Limpopo because the results of the study will be used to improve health care for adults and children with fever or headache or chills.

6. Answer all the patient's questions about this study.

7. Ask whether each identified patient will be willing to participate in the study. Explain that participation means that:
   a. The patient will receive routine care
   b. The patient may be asked to come back for follow-up

Informed consent

8. Make sure that all willing identified patients give informed consent to participating in the study. You will need to make sure that the consent form that is filled in for a willing patient is signed or has the patient's thumb print.

What should you tell patients who do not want to participate in the study ?

9. Reassure all patients who are not participating in the study (either because they do not have the above-named signs, or because they are not willing to participate) that they will receive routine health care.

What care should you provide to participating and non-participating patients ?

10. Provide optimal health care for all participating and non-participating patients

C. OBTAINING AND DOCUMENTING DATA

Filling in the form on the first visit

1. Write clearly
2. Each study form will be numbered with a clinic number and the patient identification number. . The patient study ID will be consecutively numbered

Clinic	Clinic Code	Numbers start from to
Madimbo	**MAD**	**1-500**
Mulala	**MUL**	**501-100**

3. Remember to write the date

4. Remember to write the patients date of birth as date/month/year

5. Tick all appropriate boxes

6. Record the presenting signs and symptoms (tick the "yes" column if a sign/symptom is present and "no" if it is not present)

7. Record whether an MRDT was done (tick the yes OR no column)

8. If an MRDT was done, record the result. The test line AND the control line must be rated as 0, 1 or 3 using the standard chart provided. The results must be entered on the form as either positive or negative. All invalid tests must be repeated.

9. A thick and thin blood film must be done on each patient that has an MRDT; the slides must be labeled with the unique patient identification number. (Follow the SOP for Malaria slide preparations).

10. After recording the MRDT result, then give ALL patients a date to come back for a review / follow-up in 7 days time. You must ask the patient to come back, even if his /her malaria test was negative.

11. If no malaria test was done, then do not ask the patient to come back.

12. Emphasize that it is very important for the patient to come back so that he can be managed appropriately.

13. Explain that even if the patient is feeling better on his appointment date, he/she should still come back.

14. Explain that the patient should come back immediately if he/she is feeling sicker i.e. if his symptoms get worse, or if his fever persists or if he is vomiting everything or has convulsions, or if he is unable to drink or if he starts breathing faster or has difficulty breathing. Patients who are getting sicker should not wait for their 7-day follow-up to return to the clinic.

15. Record all the treatments that you gave to the patient

16. Thank the patient and, if you have asked them to come back for follow-up, then reassure them that you are looking forward to seeing then on follow-up. Remember that only patients without MRDT test results do not come back. All patients with MRDT test results (positive or negative) should be asked to come back to the clinic for follow-up.

Filling in the follow-up form (page 2):

1. Write clearly

2. Remember to write the date

3. Remember to write the patients date of birth as date/month/year

4. Tick all appropriate boxes

5. Record the Outcome (sick or hospitalized) presenting signs and symptoms (tick the "yes" column if a sign/symptom is present and "no" if it is not present)

6. Record whether an MRDT was done (tick the yes OR no column)

7. If an MRDT was done, record the result

8. If the result is positive then take a specimen of blood and make a slide. (Follow the SOP for malaria slide preparation)

9. The test line and the control line must be rated as 0, 1 or 3 using the standard chart provided. The results must be entered on the form as either positive or negative. All invalid tests must be repeated.

10. A thick and thin blood film must be done on each patient that has an MRDT; the slides must be labeled with the unique patient identification number. The patients unique identification number must be accompanied by a capital "R" to indicate that it is a repeat. E.g. 50R or 501R
Check if patients have returned for follow-up

11. Every week check whether each patient has returned for follow-up.
For all patients that do not come back to the clinic for follow-ups, make a copy of their data collection form and send it to the malaria team (name the person / give details etc>) so that the local malaria team can follow them up at home. When the teams retrieve their information data should be recorded in the original form

General
Each clinic will have 4 files, one for each form; one file will have a blank form for the accuracy study, whilst the second will have the completed forms for the MRDT study. The third file will have the slide referral forms, which must accompany the slides when sent to the malaria teams. The fourth file will be for obtaining informed consent. All the files must be locked in a cupboard when the field officer leaves for the afternoon.

STUDY

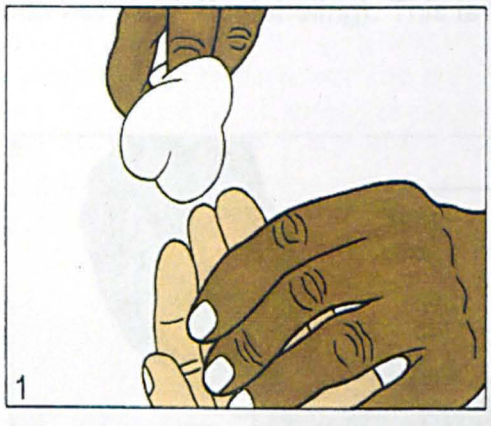
Evaluating the performance of ICT Pf Malaria Rapid Diagnostic Test, in Limpopo

Standard Operating Procedure for making thick and thin Blood films for Malaria Microscopy:

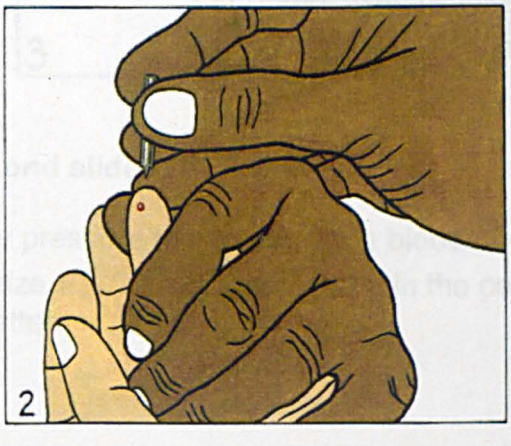
After the patient's information is recorded on the patient information form, make the blood films as follows:

1. With the patient's left hand palm facing upwards, select the third finger from the thumb (the big toe can be used for infants). **Never use the thumb for adults and children.**

2. Clean the patients finger with a alcohol swab, then dry the finger with cotton wool. Use firm strokes to increase blood circulation.



With a sterile lancet puncture the ball of the finger.



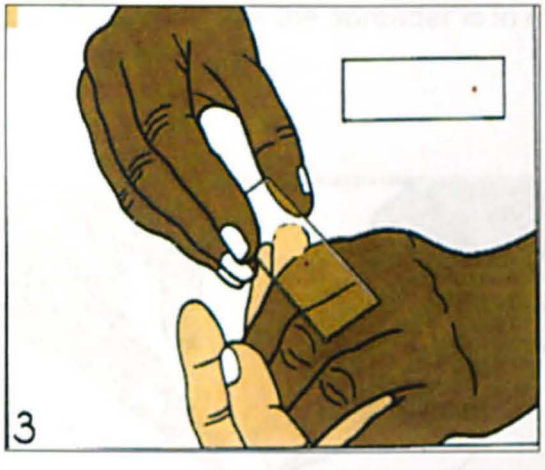
4 By applying gentle pressure to the finger express the first drop of blood

and wipe it with a dry cotton wool swab. Make sure no cotton wool remains on the finger.

5. Remove 2 blood slides from the slide box and label them with the same Number. The number should correspond with the number at the top of the patient information and MRDT results forms. One slide will be for the thin film and the other for the thick film. Make sure the number on the slide corresponds with that of the patient study ID number. Eg. MUL 050 for a patient from Mulala Clinic or MAD 100 for Medico clinic.

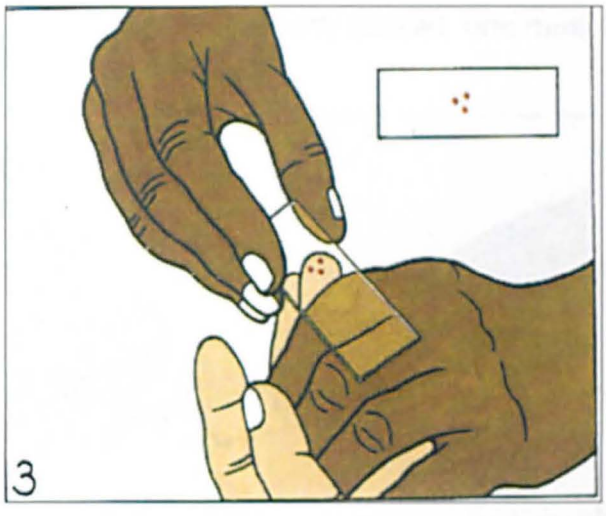
6. Working quickly and handling the glass slides only at the edges make the blood slides as follows:

   For the first slide: The Thin film
   Apply gentle pressure to the finger and collect a small drop of blood, (about this size •) one centimeter from the edge of the glass (make sure that it does not touch the labeling). This is for the thin film.



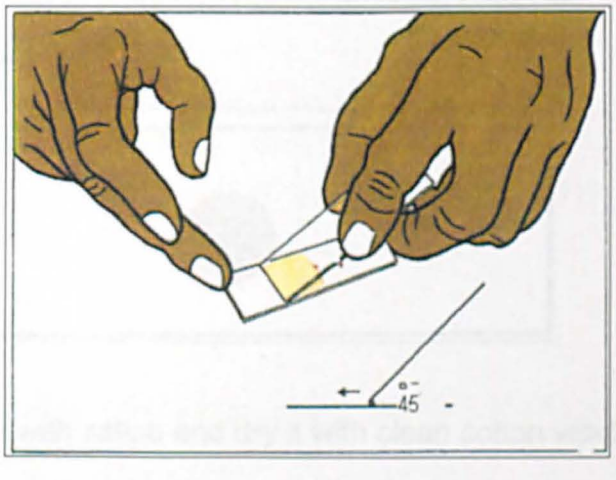
   For the second slide: The Thick Film

   Apply further pressure to express more blood and collect 3 large drops (about this size ●). Separate each drop in the center of the slide in a triangular pattern.



6. Wipe the remaining blood away from the finger.

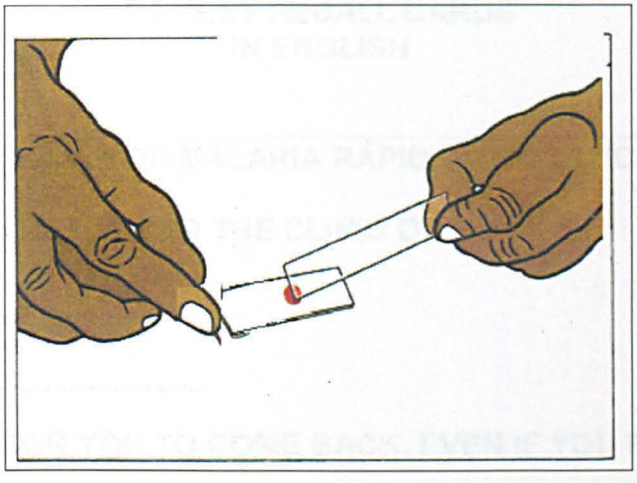
7. **THIN FILM**: using another clean slide as a "spreader" and with the blood drops resting on a flat, firm surface, touch the small drop of blood with the spreader and allow the blood to run along its edge, keeping the spreader at 45 degree angle. Make sure that the spreader is in even contact with the blood when it is spread.



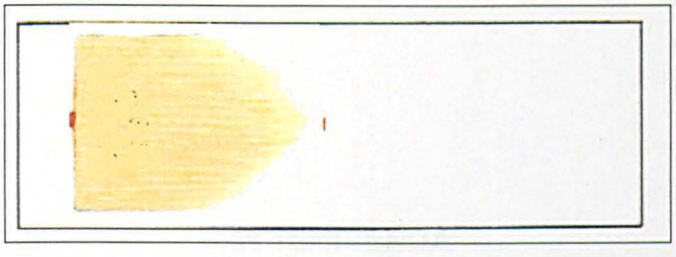
8. **THICK FILM**, always handle slides at the edges or by corner and make the thick film as follows:

   Using the corner of the spreader, quickly join the drops and spread them in a 1cm diameter circle to make a thick film. The blood must not be spread for too long, using a circular movement spread for 3-6 times.

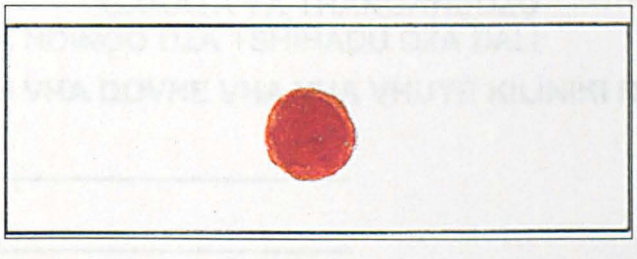
For the thick film to be correctly spread, one must be able to read a typed document through it.



Examples of a Good Thin Film:



Examples of a Good Thick Film:



9. Wash the spreader with saline and dry it with clean cotton wool, for every new patient.

10 Stick the blood slides with selo tape at the back of the patient microscopy result forms. The blood films must be on the inside of the form and the selo tape at the back of the slide.

11. keep the forms with the slides in designated box for collection by the malaria teams.

9.7 Appendix 7: Patient Recall Cards

PATIENT RECALL CARDS
IN ENGLISH

STUDY ON: EVALUATION OF MALARIA RAPID DIAGNOSTIC TESTS

PLEASE CAN YOU RETURN TO THE CLINIC ON:

DATE: _____

TIME: _____

IT IS IMPORTANT FOR YOU TO COME BACK, EVEN IF YOU FEEL BETTER

IN TSHIVENDA

GARATA YA THANGANEDZO
TSEDZULUSO DZA NDINGO DZA TSHIHADU DZA DALI
RI HUMBHELA URI VHA DOVHE VHA VHA VHUYE KILINIKI NGA:

DATUMU _____

TSHIFHINGA _____

NDI ZWA NDEME URI VHA DOVHE VHA VHUYE NAHO VHA TSHI PFA VHA KHWINE

9.8 Appendix 8: Patient Data Sheets:

Accuracy Component Form 1 Page 1

where appropriate, tick the most appropriate box

Evaluating the Performance and Usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Data Collector: Name / Surname and qualifications

_____

Clinic ID No:_____	

Patient Study ID
No._____

Date __/___/___ _ __ ___ Time.....
dd mm yyyy

PATIENT DETAILS

	M	F
Sex		

Name _____ Date of Birth___/ / ___ AGE _____ (in yrs)
Address_____ dd/mm/yr

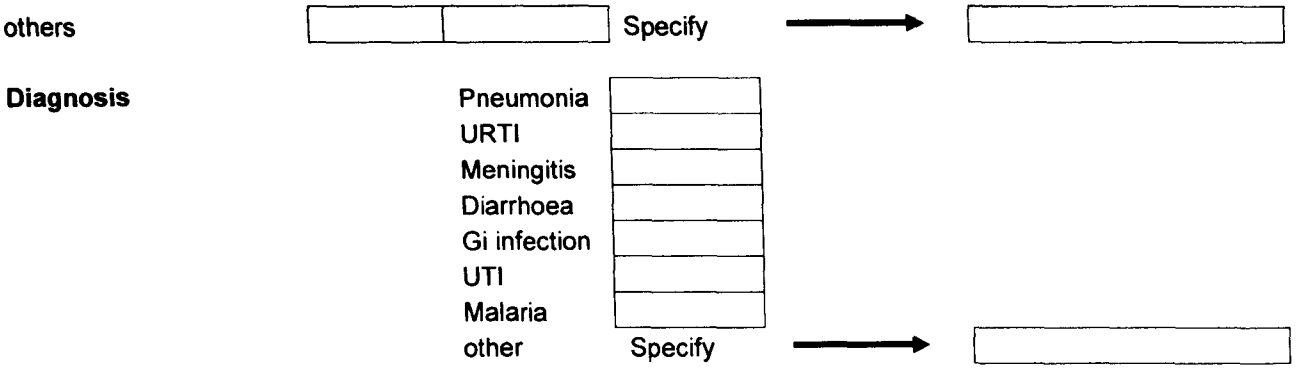
Clinic Symptoms

	NO	Yes	Duration of illness in days
Fever			
Chills			
Sweating			
Headache			
Others			

Temperature_____deg

Treatment

	NO	Yes
ACT/ Coartem®		
Primaquine		


others
Specify
Diagnosis
Pneumonia
URTI
Meningitis
Diarrhoea
Gi infection
UTI
Malaria
other
Specify

Accuracy Component Form 1, Page 2

PATIENT FOLLOW-UP STUDY

where appropriate, tick the most appropriate box

Evaluating the Performance and Usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Patient Study ID No._____ Date __/___/___ _ __ ___ Time.....

	NO	Yes	
Recovery			
Sick			
Hospitalised			if yes specify reasons for hospitalisation _____ _
Death			if yes specify reasons for death _____ _

What where the symptoms if Sick, Hospitalised or dead:

	No	Yes
Fever		
Chills		
Sweating		
Headache		
Others		

if patient is sick and has either fever, chills, sweating or headache repeat MRDT, record on FORM 3

Malaria Rapid Test kit Result Form 2

Evaluating the Performance and Usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Clinic ID No:_____

Patient Study ID No._____ Date __/___/___ _ __ ___ Time.....

dd mm yyyy

Time Blood taken_____

Test Line	Control Line

Use the standard chart provided to rate the test and control lines Ratings:
(0) = No band; (1+) = weak Band (3+) strong Band

	Negative	Positive
Results		

REPEAT Malaria Rapid Test kit Result Form 3
Evaluating the Performance and Usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Make a thick and thin blood film of all RDT study patients, and carefully label the slides with the study ID above

Clinic ID No:_____
_____

Time.....
.....
.....

Patient Study ID
No._____

Date __/___/___ _ __ ___
dd mm yyyy

Time Blood taken_____

Test Line	Control Line

Use the standard chart provided to rate the test and control lines
Ratings:(0) = No band; (1+) = weak Band (3+) strong Band

	Negative	Positive
Results		

Microscopy Result form 4

Evaluating the Performance and Usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Microscopist: Name / Surname and qualifications

_____

Clinic ID No:_____

Patient study ID No._____

Time slide stained

_____

_____.....

...

Date _of Microscopy_/___/___ _ __ ___

Time Blood taken_____

	Neg	Pos	
Microscopy results			if positive: ID Species*_____*P.* _____

*** if positive Calculate parasite density**

Asexual Parasites Counted [ ]

___

___ X 8000 WBCμ-1 [ ] = [ ] Parasites μ-1

WBC counted

Sexual Parasites Counted [200] [ ]

___

___ X 8000 WBCμ-1 [ ] = [ ] Parasites μ-1

WBC counted [200]

9.9 Appendix 9: Participant information sheet and informed consent: Field accuracy study (Component 1)

Information sheet:

PARTICIPANT INFORMATION SHEET

TITLE OF RESEARCH PROJECT: Evaluating the performance and usage of ICT *Pf* Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Prinicipal Investigator:
Devanand Moonasar: National Department of Health South Africa and London School of Hygiene & Tropical Medicine, UK

Contact Information:
Tel: +27 (0) 12 3120102
Fax: +27(0) 12 3123113
e-mail: moonad@health.gov.za/patrick.moonasar@lshtm.ac.uk

WHY ARE WE DOING THIS STUDY?

Malaria is a disease that can be treated and cured. Early diagnosis and treatment is important for preventing severe or complicated malaria and death. Since Rapid Malaria Tests are being used for Malaria diagnosis in your area, it is important for these tests to be accurate and easy to use. We did a study in March 2006 and the key findings from that study indicated that there were key concerns with the accuracy of the Malaria Rapid Diagnostic test. We are therefore doing this study to determine the accuracy of Rapid diagnostic tests under field conditions.

DO I HAVE TO TAKE PART?

It is entirely up to you to decide whether or not to take part in this study. If you decide not to take part, this will not affect you in any way. If you do decide to take part you will asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

IF I TAKE PART IN THIS STUDY, WHAT WILL HAPPEN?

After you have had a chance to ask questions, and have signed the informed consent form, we will take your blood and test you for malaria. If you do not give permission, we will still test you for malaria but will not include you in the study. The test will take 15 minutes to complete. We will make a slide from your blood and may take some of your blood in a little tube for testing. We may ask you to return, to see if you are well. If you are not well, we will do more tests and give you medicine if needed You can decide to withdraw from the study if you like and if you are uncomfortable then feel free to ask questions.

WHAT ARE THE RISKS AND BENEFITS OF TAKING PART IN THIS STUDY?
There are no risks in taking part in this study, but we would ask for about 15 minutes of your time today. This study may not benefit you right now, but the results of the study will help us to know what the accuracy of the Rapid Diagnostic tests are and how we might improve this. It will also help sisters in this clinic and health centres to be confident with the use and results of the Rapid Diagnostic tests. If they are satisfied with the results you do not have to go to the he hospital for retesting.

WHAT ARE THE COSTS TO ME?
There is no cost to you for participating in the study. If you agree to the study then you need to sign a consent form.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?
All information collected, as part of this study will be kept securely and confidentially. Mr Devanand Moonasar the lead researcher for this study will be responsible for this. You will not be personally identified in any report about this study. All information will be kept in a locked and secured cabinet.

WHAT IF I HAVE MORE QUESTIONS I WISH TO ASK ABOUT THIS STUDY?
If you have any questions about this study, please ask us now. We will also give you a copy of this information sheet, which explains the study to take away with you. If you have questions later you can telephone Mr. Devanand Moonasar on 012 312 0102. The committees giving ethical approval for this study are the Research Ethics Committees of the London School of Hygiene and Tropical Medicine, UK and the Medical University of South Africa.

9.10 Appendix 10: Tshivenda translation:

THOHO YA PHUROJEKE

U lungulula ku shumele na ku shumisele kwa (ICT Pf Malaraia) Ndingo ya tshihadu Limpopo Africa Tshipembe.

NDI NGANI RI TSHI TEA U ITA NDINGO ?

Dali ndi vhulwadze vhune hanga ilafhea ha do vha ha fhola
U tavhanya u wanala na u ilafha ndi zwa ndeme u thivhela u hulela ha Dali na lufu (kana u lovha).
U bva tshe ha thoma u shumiswa ndingo dza tshihadu dza vhulwadze ha Dali hu u itela u wana u vha hone ha vhulwadze ha Dali kha vhupo ha havho, Ndi zwa ndeme uri ndingo hedzi dzi vhe dzi vhe kwadzo na u shumisea nga u leluwa
Ndi ngazwo ri tshi tea u divha vh di ha ku shumele kwa ndingo idzi.

NDI NGA DZHENELA NAA ?

Zwi bva kha vhone vhane uri vha a funa u dzhenelela kha tsedzuluso idzi. Arali vha tshi Pfa vha sina dzangalelo la u dzhenelela izwo a zwi nga vha vhangeli thaidzo hunwe fhethu. Arali vha vha na dzangalelo la u dzhenelele, vha do humbelwa u saina fomo ya u sumba uri vhone vha na dzangalela. Vhana ndugelo dza u sa tsha isa phanda na tsedzuluso idzi tshifhinga tshinwe na tshinwe

HU DO ITEA MINI ARALI NDA DZHENELELA TSEDZULUSO

Nga murahu ha musi vho wana tshifhinga tsha u vhudzisa mbudziso na u saina fomo ya tsumbo dzangalelo, Ri do dzhia malofha a vho ra vha lingulula Dali. Arali vha sa ri nea thendelo ri do vha dzhia malofha u lingula Dali fhedzi a vha nga vhi murado wa tsedzuluso. Ndingo dzi do dzhia minethe ya fumi na mitanu u fhela. Ri do dzhia malofha avho ra ita tshilaidi (u rothisela malofha kha ngilasi) ra dovha ra rothisela shotha kha tshipida tha Bambiri. Vha nga kha di di bvisa kha tsedzuluso idzi arali vha tshi funa nahone vha tshi Pfa vha songo dzudzanyea kha vha vho fholowe u vhudzia mbudziso.

VHUDI NA VHUVHI HA U DZHENELELA TSEDZULUSO

A huna khombo kha u di dzhenisa kha tsedzuluso fhedzi ri do humbela mithethe ya fumi na mitanu kha tshifhinga tshavho namusi. Hedzi tsedzuluso zwi nga itea dza sa vha vhuyedze tshithu zwa zwino fhedzi mvelelo dza tsedzuluso dzi do ri thusa u di vha vhukoni ha dingo dza tshihadu na uri ri nga khwinifhadza hani, Zwi do dovha zwa tshusa vhaongi vha ino kiliniki na dzi Health Centres u vha na u fhulufhela kha u shumisa na mvelelo dza ndingo dza tshihadu, Arali vhaongi vha fushea nga Mvelelo dza ndingo , A vho nga tsha tea uya sibadela u dovholola ndingo.

MBADELO KHA NNE NDI DZI FHIO ?
A huna mbadelo musi vha tshi dzhenela kha tsedzuluso. Arali vha tshi tendelana na tsedzuluso vha tea u saina fomo ya u di dzhenisa

U DZHENELE HANGA TSEDZULUSO ZWI DO VHA TSHI DZUMBE NAA ?
Vhutanzi hothe ho kuvhanganywaho sa tshipida tsha tsedzuluso vhu do vha tsireledzeaho ha dovha ha vha ha tshipiri. Vho Devanand Moonasar, muhulwane wa tsedzuluso heyi, vha do vha vhone muthu a hwalalo vhu di fhinduleli hothe.

Zwidodombedzwa nga ha vhone a zwi nga andazwi kha ripoto inwe na inwe nga tsedzuluso idzi. Vhutanzi hothe nga ha tsedzuluso idzi vhu vhewa ha kwinetshelwa fhethu ho tsireledzeaho.

ZWINO ARALI NDI ND DZINWE MBUDZISO NDI TSHI TAMA U VHUDZISA NGA HA TSEDZULUSO NI ?
Arali vha na dzinwe mbudziso, vhanga founela vho Devanand Moonasar kha 012 312 0102. Dzi komiti dzi neaho tendelo dza Pfanelo dza vhuthu dza tsedsuluso idzi ndi komiti ya tsedzuluso ya Pfanelo dza vhathu ya tshikolo tsha mutakalo na zwa mishonga ya Tropika tsha London, UK na gudedzi la mishonga na dzilafho la Afrika Tshipembe.

9.11 Appendix 11: Consent Form Field accuracy study

CONSENT FORM

Title of project: Evaluating the performance and usage of ICT *Pf* Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Investigator:
Devanand Moonasar: National Department of Health South Africa and London School of Hygiene & Tropical Medicine, UK

CONSENT TO PARTICIPATE IN THE RESEARCH STUDY

- I have read the information sheet about this study and I understand what will be required of me and what will happen to me if I take part in the study.
- I understand that I may withdraw from this study at any time without giving a reason and without affecting my normal care and management.
- I agree to take part in the study **YES / NO** (answer to be circled)

Participant's signature/ Thumb print :_____

Date: _____

Participant's name: _____

Informed Consent, Thivenda Translation;

Patient ID No_____

Fomo ya thendelo ya tsedzululo dza vhukoni kha mupo

FOMO YA U NEA THENDELO
Dzina la tshipida tsha mushumo wa tsedzuluso: U SEDZULUSA MASHUMELE NA KU SHUMISELE KWA ICT Pf MALARIA, NDINGO YA U WANULUSA YA TSHIHADU

THENDELO YA U DZHENELELA KHA NGUDO TSEDZULUSI.

1.Ndo vhala vhutanzi kha bambiri nga ha tsedzuluso idzi nahone ndi Pfesesa zwine zwa todea kha nne na zwine zwa do todea kha nne na zwine zwa do bvelela kha nne musi ndi tshi dzhia tshipida kha tsedzuluso idzi.

Ndi Pfesesa uri ndi nga di bvisa kha tsedsuluso idzi tshifhinga tshinwe na tshinwe ndi songo nea tshiitisi zwine zwa sa do kwama ku togomelelwe kwa Kwanga Ndi a tenda u dzhia tshipida (dzhenelela) kha tsedzuluso idzi
EE ! HAI
(Phindulo i tangeledzwe)

TSAINO YA MUDZHELEO / U GANDISA GUNWE _____

DATUMU _____

MADZINA A MUDZHENELELI _____-

9.12 Appendix 12: Clinics and Health Centres selected for testing

End-user performance:

District	Municipality Name	Clinic Name	Malaria Cases				
			2002	2003	2004	2005	Average
VHEMBE	MUSINA	Tshiungani Clinic	13	12	6	37	10.3
VHEMBE	THULAMELA	Shikundu Clinic	18	16	6	10	13.3
VHEMBE	THULAMELA	Duvhuledza Clinic	13	21	7	13	13.7
VHEMBE	THULAMELA	Sterkstroom Clinic	10	25	9	8	14.7
VHEMBE	THULAMELA	Shingwedzi Clinic	21	45	13	13	26.3
VHEMBE	THULAMELA	Ntlhaveni E Clinic	31	14	14	10	19.7
VHEMBE	THULAMELA	Ntlhaveni D Clinic	12	8	15	12	11.7
VHEMBE	THULAMELA	Mukula clinic	9	18	15	10	14
VHEMBE	THULAMELA	Tshaulu Clinic	37	22	16	5	25
VHEMBE	THULAMELA	Sambandou Clinic	27	28	22	24	25.7
VHEMBE	MUTALE	Mutale Health Centre	37	40	23	11	33.3
VHEMBE	MAKHADO	Khomela Clinic	1	4	30	0	11.7
VHEMBE	THULAMELA	Makuleke clinic	78	101	31	48	70
VHEMBE	THULAMELA	Mhinga Clinic	99	129	32	59	86.7
VHEMBE	THULAMELA	Lambani Clinic	52	57	41	51	50
VHEMBE	MUSINA	Nancefield Health Centre	18	21	42	9	27
VHEMBE	MUTALE	Folovhodwe Clinic	125	103	60	76	96
VHEMBE	MUTALE	Makuya Clinic	56	84	77	90	72.3
VHEMBE	MUTALE	Tshipise Clinic	87	70	96	61	84.3
VHEMBE	MUSINA	Madimwo/Madimbo Clinic	146	202	121	104	156.33
VHEMBE	MUTALE	Masisi Clinic	126	192	157	123	158.3
VHEMBE	MUTALE	Mulala clinic	157	239	168	164	188

9.13 Appendix 13: Check list for end-user observations.

Table B: Interpretation of results

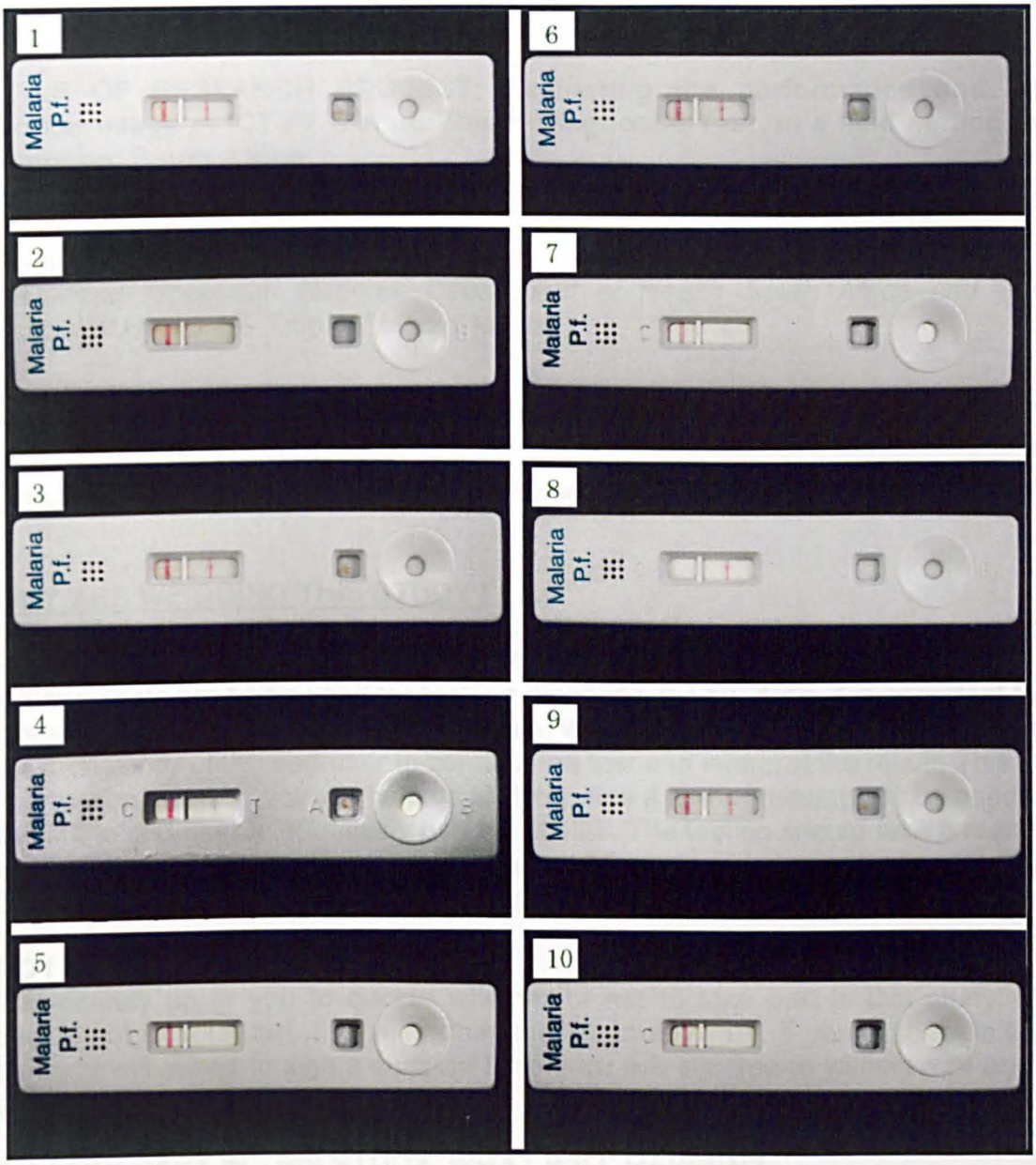
Table A: Observation of tests

Observer	Date observed	User #	User Sex	User Age	Education	Malaria tx experience?	RDT Use?
			[1] M		[1]	[1] Yes	[1] Yes
		User First Name			[2]	[2] No	[2] No
Site	Day Month Year		[2] F		[3]	How many months?	How many months?
					[4]		

Observation Number	1			2			3			Comments
Was this test done on a real patient? *Circle the correct answer: 1=Yes 2=No*	1 Y	2 N		1 Y	2 N		1 Y	2 N		ASK at end- if they knew
Was patient febrile? *Circle the correct answer: 1=Yes 2=No 3=Not applicable (if not a real patient)*	1 Y	2 N	3	1 Y	2 N	3	1 Y	2 N	3	
For each step below, circle 1 if the HW performed the step correctly, circle 2 if the HW performed the step incorrectly, circle 3 if the HW skipped the step										
1. Assemble new test packet, swab, lancet & gloves.	1	2	3	1	2	3	1	2	3	Date Y N
2. Put on new pair of gloves.	1	2	3	1	2	3	1	2	3	
3. Check expiry date on test package to make sure test is still valid.	1	2	3	1	2	3	1	2	3	
4. Write patient's name on device	1	2	3	1	2	3	1	2	3	
5. Clean finger with alcohol.	1	2	3	1	2	3	1	2	3	
6. Allow finger to dry before pricking it.	1	2	3	1	2	3	1	2	3	
7. Using a sterile lancet, puncture the side of the ball of the finger.	1	2	3	1	2	3	1	2	3	
8. Dispose of lancet in sharps bin immediately after pricking finger.	1	2	3	1	2	3	1	2	3	
9. **Touch the tip of the tube to the blood until the tube is half full**	1	2	3	1	2	3	1	2	3	
10. Using the loop, immediately touch the tip of the tube with blood in the smaller hole	1	2	3	1	2	3	1	2	3	
11. Dispense 5 drops of clearing buffer into the larger hole	1	2	3	1	2	3	1	2	3	
12. Wait 15 minutes before reading results.	1	2	3	1	2	3	1	2	3	
13. Read test results correctly.	1	2	3	1	2	3	1	2	3	
14. Record results in CHW register.	1	2	3	1	2	3	1	2	3	
15. Dispose of gloves, wrappers, alcohol swab, loop, desiccant & cassette in non-sharps container.	1	2	3	1	2	3	1	2	3	
Row Total:			/15			/15			/15	

Battery #	1	2	3	4	5	6	7	8	9	10
Test result	*(Write an "X" in the appropriate box below each test number to indicate if the HW interprets the test result as "Positive" "Negative" or "Ambiguous")*									
Positive										
Negative										
Ambiguous										

9.14 Appendix 14:Colour chart for Interpreting MRDT results



9.15 Appendix 15: Participant information sheet: End-user ability to conduct the test and interpret the results

PARTICIPANT INFORMATION SHEET

TITLE OF RESEARCH PROJECT: Evaluating the performance and health worker usage of ICT *Pf* Malaria Rapid Diagnostic Test, in a field setting in the Limpopo, South Africa

Prinicipal Investigator:
Devanand Moonasar: National Department of Health South Africa and London School of Hygiene & Tropical Medicine, UK

Contact Information:
Tel: +27 (0) 12 3120102
Fax: +27(0) 12 3123113
e-mail: moonad@health.gov.za/patrick.moonasar@lshtm.ac.uk

WHY ARE WE DOING THIS STUDY?

Malaria is a disease that can be treated and cured. Early diagnosis and treatment is important for preventing severe or complicated malaria and death. Since Rapid Malaria Tests are being used for Malaria diagnosis in your area, it is important for these tests to be accurate and easy to use. We are doing this study to determine the proficiency of the end-user to conduct the test and interpret the result. This information given by you will help us to determine if further training will be needed to assist the end-user to effectively use the MRDT. The testing should take a maximum of 45 minutes. We are planning to test about 20 users who are involved with Rapid Diagnostic tests for diagnosis and management of malaria.

DO I HAVE TO TAKE PART?

It is entirely up to you to decide whether or not to take part in this study. If you decide not to take part, this will not affect you in any way. If you do decide to take part you will asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

IF I TAKE PART IN THIS STUDY, WHAT WILL HAPPEN?

After you have had a chance to ask questions, and have signed the informed consent form, you will conduct 2 MRDTs and interpret the results, whilst being observed and we will take notes on paper.

WHAT ARE THE RISKS AND BENEFITS OF TAKING PART IN THIS STUDY?

There are no risks in taking part in this study, This study may not benefit you right now, but the results of the study will help us to know what the level of skills are to

conduct and interpret the results of an MRDT and how we may improve the training of the end-user for your setting.

WHAT ARE THE COSTS TO ME?

There is no cost to you for participating in the study.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

All information collected, as part of this study will be kept securely and confidentially. Mr Devanand Moonasar the lead researcher for this study will be responsible for this. You will not be personally identified in any report about this study. All information will be kept in a locked and secured cabinet.

WHAT IF I HAVE MORE QUESTIONS I WISH TO ASK ABOUT THIS STUDY?

If you have any questions about this study, please ask us now. We will also give you a copy of this information sheet, which explains the study to take away with you. If you have questions later you can telephone Mr. Devanand Moonasar on 012 312 0102. The committees giving ethical approval for this study are the Research Ethics Committees of the London School of Hygiene and Tropical Medicine, UK and the Medical University of South Africa.

9.16 Appendix 16 : Consent form End-user ability to conduct the test and Interpret the results

CONSENT FORM

Title of project: Evaluating the performance and health worker usage of ICT Pf Malaria Rapid Diagnostic Test, in a field setting in the Limpopo, South Africa

Investigator:
Devanand Moonasar: National Department of Health South Africa and London School of Hygiene & Tropical Medicine, UK

CONSENT TO PARTICIPATE IN THE RESEARCH STUDY

- I have read the information sheet about this study and I understand what will be required of me and what will happen to me if I take part in the study.

- I understand that I may withdraw from this study at any time without giving a reason and without affecting my normal care and management.

- I agree to take part in the study **YES / NO** (answer to be circled)

Participant's signature :_____

Date: _____

Participant's name: _____

9.17 Appendix 17: Quality Assurance study sites

DISTRICT	MUNICIPALITY	FACILITYNAME	2002	2003	2004	Average
VHEMBE	MAKHADO	Louis Trichardt Hospital	13	21	2	12
VHEMBE	MAKHADO	Khomela Clinic	1	4	30	11.7
VHEMBE	MAKHADO	Elim Hospital	71	103	61	78.3
VHEMBE	MUSINA	Tshiungani Clinic	13	12	6	10.3
VHEMBE	MUSINA	Nancefield Health Centre	18	21	42	27
VHEMBE	MUSINA	Madimbo Clinic	146	202	121	156.3
VHEMBE	MUSINA	Messina Hospital	336	307	315	319.3
VHEMBE	MUTALE	Folovhodwe Clinic	125	103	60	96
VHEMBE	MUTALE	Tshipise Clinic	87	70	96	84.3
VHEMBE	MUTALE	Manenzhe Clinic	16	22	17	18.3
VHEMBE	MUTALE	Matavhela Clinic	14	33	35	27.3
VHEMBE	MUTALE	Donald Fraser Hospital	267	420	293	326.6
VHEMBE	THULAMELA	Shikundu Clinic	18	16	6	13.3
VHEMBE	THULAMELA	Shingwedzi Clinic	21	45	13	26.3
VHEMBE	THULAMELA	Ntlhaveni E Clinic	33	16	17	22.0
VHEMBE	THULAMELA	Ntlhaveni E Clinic	31	14	14	19.6
VHEMBE	THULAMELA	Ntlhaveni D Clinic	12	8	15	11.7
VHEMBE	THULAELA	Mukula clinic	9	18	15	14
VHEMBE	THULAMELA	Dzwerani Clinic	14	10	6	10
VHEMBE	THULAMELA	Vhurivhuri Clinic	20	29	8	19
VHEMBE	THULAMELA	Lambani	18	20	7	15
VHEMBE	THULAMELA	Siloam	14	13	6	11
VHEMBE	THULAMELA	Malamulele Hospital	129	378	280	262.3

9.18 Appendix 18 Data recording sheet for QC study:

Quality Control Result form 5

Tick Appropriate boxes

Evaluating the Performance and Usage of ICT Pf Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Clinic ID No:_____

Clinic ☐

LAB ☐

Date __/__/___ _ __ ___ Time.....

dd mm yyyy

Nearest Hospital Lab_____

Distance from Lab_____kms____

Average Clinic Temp:_____ Deg

	none	Fair	Good	Excellent
Experience with using MRDTs				

Storage period of PCW ----------days

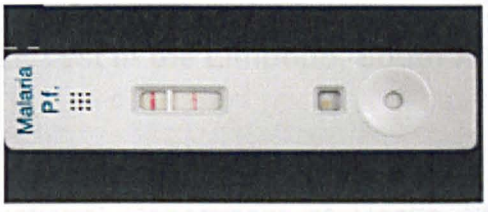
MRDT results

		Sample 1	**Sample 2**
	MRDT Batch ID		
	Negative		
	Positive		
if positive	barely visible		
	weakly positive		
	strong positive		
	PCW results		
	Negative		
	Positive		
if positive	barely visible		
	weakly positive		
	strong positive		

Sample 1= Blood Sample, Sample 2 = positive control well sample (PCW). Clinics should only fill in results for Sample 2.

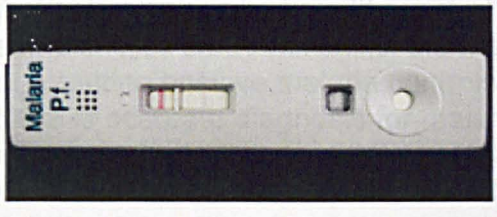
9.19 Appendix 19 Standardized Charts for interpreting line intensity of positive quality controls for MRDTs.

Strong Positive

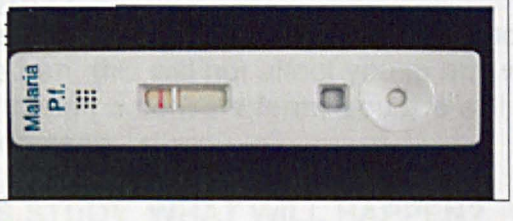


Weak Positive

Barely visible Positive



Negative:



9.20 Appendix 20: Participant Information sheet: Quality assurance for routine sensitivity monitoring of HRP II antigen

PARTICIPANT INFORMATION SHEET

TITLE OF RESEARCH PROJECT: Evaluating the performance and of ICT *Pf* Malaria Rapid Diagnostic Test in the Limpopo, South Africa

Prinicipal Investigator:
Devanand Moonasar: National Department of Health South Africa and London School of Hygiene & Tropical Medicine, UK

Contact Information:
Tel: +27 (0) 12 3120102
Fax: +27(0) 12 3123113
e-mail: moonad@health.gov.za/patrick.moonasar@lshtm.ac.uk

WHY ARE WE DOING THIS STUDY?

Malaria is a disease that can be treated and cured. Early diagnosis and treatment is important for preventing severe or complicated malaria and death. Since Rapid Malaria Tests are being used for Malaria diagnosis in your area, it is important for these tests to be accurate and easy to use. We are doing this study to compare which setting will be most effective to conduct quality control on MRDT through the use of a positive control sample. This study will assist the malaria control programmes to implement a routine positive malaria controls for testing MRDT, with the aim of ensuring that there is accurate diagnosis for malaria at the primary health care level. We are planning to conduct a series of MRDT tests at the district laboratory and at selected clinics. Your laboratory/clinic has been selected to be part of this study.

DO I HAVE TO TAKE PART?

Your laboratory/clinic has been selected to be part of this study.
If you decide not to take part, this will not affect you in any way. If you do decide to take part you will asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

IF I TAKE PART IN THIS STUDY, WHAT WILL HAPPEN?

After you have had a chance to ask questions, and have signed the informed consent form, we will ask you to store the Malaria HRP II positive control in your laboratory or in the clinic for up to 3 months, every month a technician from your laboratory will have to test the sample using whole blood or citrate as a diluent. Results of this test should be recorded in a data sheet.

WHAT ARE THE RISKS AND BENEFITS OF TAKING PART IN THIS STUDY?

There are no risks in taking part in this study, but the testing of the MRDT should take you approximately 30 minutes once a month for 3 months. This study may not benefit you right now, but the results of the study will help us to know which site would be optimal to run a routine MRDT quality check. It will ensure that there confidence in the results of the MRDT and there will be few referrals to hospitals as doubtful results would be resolved with a positive control.

WHAT ARE THE COSTS TO ME?

There is no cost to you for participating in the study.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

All information collected, as part of this study will be kept securely and confidentially. Mr Devanand Moonasar the lead researcher for this study will be responsible for this. You will not be personally identified in any report about this study. All information will be kept in a locked and secured cabinet.

WHAT IF I HAVE MORE QUESTIONS I WISH TO ASK ABOUT THIS STUDY?

If you have any questions about this study, please ask us now. We will also give you a copy of this information sheet, which explains the study to take away with you. If you have questions later you can telephone Mr. Devanand Moonasar on 012 312 0102. The committees giving ethical approval for this study are the Research Ethics Committees of the London School of Hygiene and Tropical Medicine, UK and the Medical University of South Africa.

9.21 Appendix 21: Consent form Quality assurance for routine sensitivity monitoring of HRP II antigen

CONSENT FORM

Title of project: Evaluating the performance and health worker ICT *Pf* Malaria Rapid Diagnostic Test, in the Limpopo, South Africa

Investigator:
Devanand Moonasar: National Department of Health South Africa and London School of Hygiene & Tropical Medicine, UK

CONSENT TO PARTICIPATE IN THE RESEARCH STUDY

- I have read the information sheet about this study and I understand what will be required of me and what will happen to me if I take part in the study.

- I understand that I may withdraw from this study at any time without giving a reason and without affecting my normal care and management.

- I agree to take part in the study **YES / NO** (answer to be circled)

Participant's signature :_____

Date: _____

Participant's name: _____

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An exploratory study of factors that affect the performance and usage of rapid diagnostic tests for malaria in the Limpopo Province, South Africa

Devanand Moonasar*[1], Ameena Ebrahim Goga†[2], John Frean†[3], Philip Kruger†[4] and Daniel Chandramohan†[1]

Address: [1]Disease Control and Vector Biology Unit, London School of Hygiene and Tropical Medicine, London, UK, [2]Southern African Fogarty AIDS International Training and Research Programme (AITRP), The Centre for the AIDS Programme of Research in South Africa (CAPRISA), Durban, South Africa, [3]Parasitology Reference Unit, National Health Laboratory Service, Johannesburg, South Africa and [4]Malaria Control Programme, Limpopo Department of Health and Social Development, Tzaneen, South Africa

Email: Devanand Moonasar* - Patrick.moonasar@lshtm.ac.uk; Ameena Ebrahim Goga - aegoga@yahoo.com; John Frean - johnf@nicd.ac.za; Philip Kruger - PKruger@dhw.norprov.gov.za; Daniel Chandramohan - Daniel.chandramohan@lshtm.ac.uk

* Corresponding author †Equal contributors

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Abstract

Background: Malaria rapid diagnostic tests (RDTs) are relatively simple to perform and provide results quickly for making treatment decisions. However, the accuracy and application of RDT results depends on several factors such as quality of the RDT, storage, transport and end user performance. A cross sectional survey to explore factors that affect the performance and use of RDTs was conducted in the primary care facilities in South Africa.

Methods: This study was conducted in three malaria risk sub-districts of the Limpopo Province, in South Africa. Twenty nurses were randomly selected from 17 primary health care facilities, three nurses from hospitals serving the study area and 10 other key informants, representing the managers of the malaria control programmes, routine and research laboratories, were interviewed, using semi-structured questionnaires.

Results: There was a high degree of efficiency in ordering and distribution of RDTs, however only 13/20 (65%) of the health facilities had appropriate air-conditioning and monitoring of room temperatures. Sixty percent (12/20) of the nurses did not receive any external training on conducting and interpreting RDT. Fifty percent of nurses (10/20) reported RDT stock-outs. Only 3/20 nurses mentioned that they periodically checked quality of RDT. Fifteen percent of nurses reported giving antimalarial drugs even if the RDT was negative.

Conclusion: Storage, quality assurance, end user training and use of RDT results for clinical decision making in primary care facilities in South Africa need to be improved. Further studies of the factors influencing the quality control of RDTs, their performance of RDTs and the ways to improve their use of RDTs are needed.

Background

The South African National Malaria treatment guidelines stipulate that malaria treatment (using artemesinin-based combination therapy) should be based on definitive diagnosis using microscopy or malaria rapid diagnostic tests (RDTs) [1]. South Africa has been implementing RDTs to diagnose malaria within malaria endemic areas since 2001[2]. In a primary health care setting, RDTs are most appropriate: they are easy to use, do not require sophisticated technology and give rapid results [3]. The functioning and accuracy of RDTs can be affected by several factors, including manufacturing defects, storage, transport, and end-user performance [4]. Malaria diagnostic tests need to be highly accurate because false negative and false positive diagnoses have medical, social, and economic consequences such as prolongation of illness, increase in morbidity and mortality and loss in credibility of health services [5,6].

The Limpopo Province, is one of three malaria endemic provinces in South Africa and has the highest malaria incidence [2]. Figure 1 provides a map showing magisterial areas. Although RDTs was introduced for malaria diagnosis in Limpopo in 2003, operational issues relating to its performance and use have not been rigorously investigated. A study was therefore undertaken in the Limpopo Province to determine which factors affected quality and usage of RDTs.

Methods

Purposefully selected key informants and randomly selected nurses at the primary health facility level were interviewed using a semi-structured questionnaire. Key informants included three hospital pharmacy staff, one regional pharmacy manager, three district malaria managers, one provincial malaria control manager and two researchers. Among the nurses interviewed, 17 were from primary health care (PHC) clinics and three were from hospitals.

A two-stage sampling procedure was used to select PHC clinic staff [7]. All clinics and health centers within the three malaria-affected districts were listed. Clinics and health centers with fewer than 10 malaria cases per annum were excluded from the sampling frame. In the first stage of sampling, 10% of the clinics (10/100) and 20% of health centers (7/35) within each selected district were randomly selected. In the second stage, one nursing sister was randomly selected from each selected health facility. Interviews were transcribed, ordered and coded in matrixes, using the key categories of procurement & stock monitoring, storage, transport, quality control and end user experiences [8].

Ethics

The University of Limpopo Research Ethics Committee, the Limpopo Department of Health and Social Development and the London School of Hygiene and Tropical Medicine granted ethical permission for this study. Informed consent was acquired from all interviewees.

Findings

Procurement and stock monitoring

In all health facilities, there was evidence that stocks and expiry dates were monitored regularly. Stock monitoring methods ranged from stock cards (paper based) to electronic systems. Nursing staff were aware of the seasonal increase in malaria cases and stated that they ordered more RDT stock before and during the season; however, only 20% of the nurses interviewed were able to accurately give the limits of the malaria season (September-May). More than half (55%) of the nurses indicated that stock-outs of RDTs occurred. However, they reported that this was rare (one or two times in a season) and contingency plans existed to replace stock from either the nearest clinic or hospital pharmacies. Replacement of stock took place within 24 hours. District malaria managers reported RDT stock-outs in clinics in the 2005/2006-malaria season. They responded by alerting the necessary authorities (district hospital or regional pharmacy depots) or transporting malaria kits to facilities.

Pharmacy staff reported deploying pharmacy assistants to some health facilities to assist with ordering of pharmaceuticals including RDTs. One hospital had a bar code system for ordering pharmaceutical supplies. At the regional pharmacy level an electronic system is in place to increase stock as demand increased, and in most cases this is proportional to the seasonal increase in malaria cases.

Storage of RDTs

Sixty five percent (13/20) of the nurses reported that RDTs were stored correctly i.e. in an air-conditioned room with regular temperature monitoring. Among the nurses from the seven facilities that did not implement correct storage, three (42%) were very concerned with temperature fluctuation as thermometers were unavailable for temperature monitoring. The remaining 4/7 (53%) indicated that although their clinics lacked air conditioning, room temperature was monitored, and that it rarely rose above 30°C. This however was not corroborated with any recorded data.

Malaria managers (district and provincial) accepted that some clinics did not keep RDTs in a cool environment; however they were not concerned about this, commenting that "*the kits did not stay in the clinics for too long*", due to their frequent use.

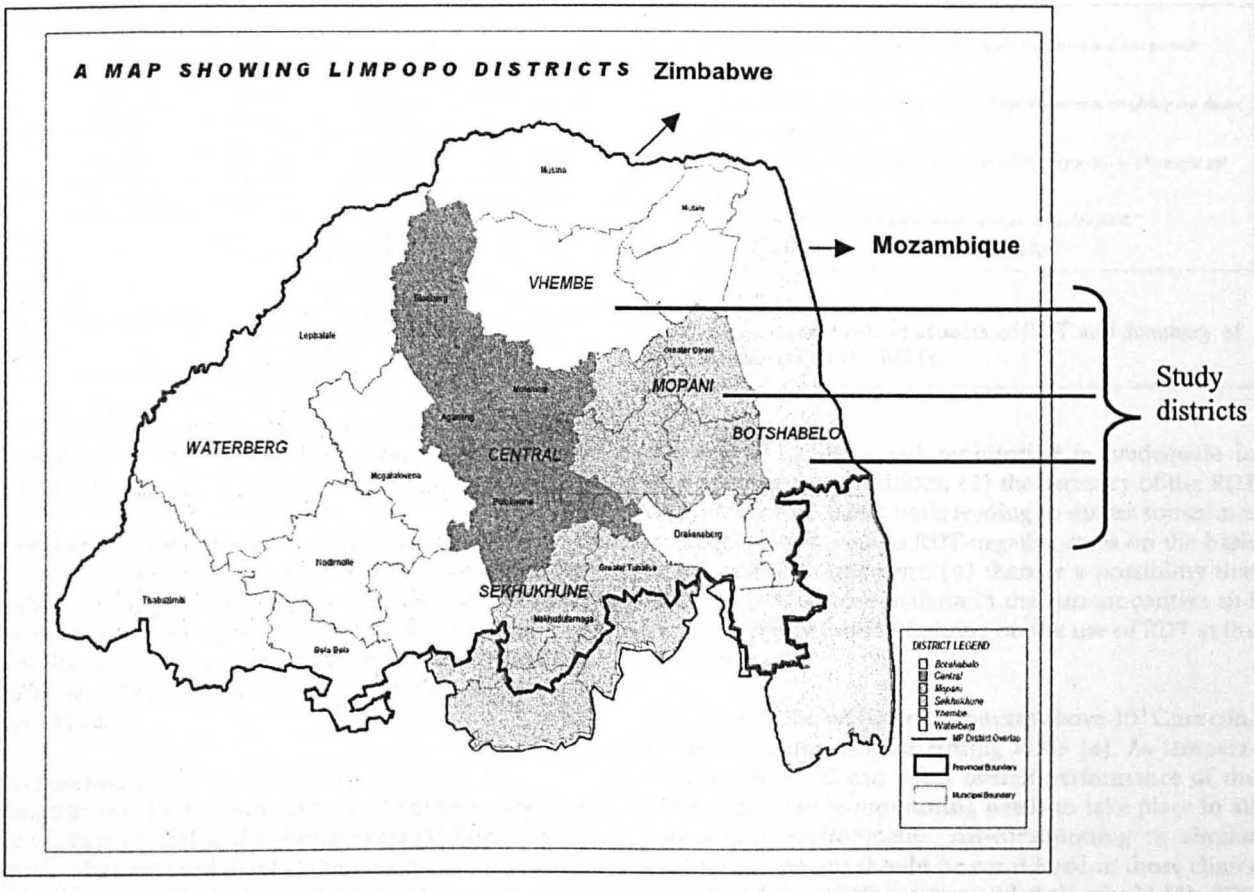

Figure 1
Magisterial Districts of the Limpopo Province.

Pharmacy managers in hospitals and regional depots stored RDTs in an air-conditioned environment (temperature range 15–25°C). Monitoring charts were produced when requested by the interviewer.

Quality control of using RDTs

Only 4/20 nursing staff said that they checked the quality of RDTs, this they reported to have been done, by comparing the agreement between a diagnosis based on clinical signs and symptoms and the RDT result. Two of 20 nurses reported that they used blood smear results to confirm the RDT results occasionally. Three (15%) nurses reported that they gave antimalarial drugs to RDT negative patients if the clinical presentation was suggestive of malaria.

Figure 3 highlights the key challenges identified by nurses, researchers and malaria managers relating to quality.

In summary, managers were very concerned that RDTs quality were not being monitored at health facility level. One informant stated, "*RDT quality control, both at the manufacturing side and at the testing stage, was lacking. The key is the end user's ability to distinguish between positive and negative results.*"

End User experiences in using RDTs

Nursing staff had huge praise for the use of the RDTs, Figure 4 highlights nurses' responses on positive aspects of using the RDTs.

More than half the nurses (12/20) however reported that they did not receive external training on RDT use; however, 10 nurses reported receiving in-house training. Almost all (19/20) nurses said that they were confident in using the RDT.

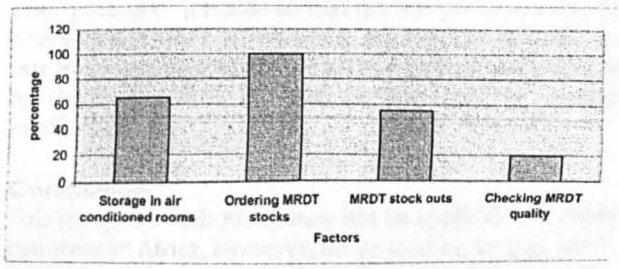

Figure 2
Graph Showing factors affecting the quality and usage of RDTs.

The accuracy of RDT result was the key concern for 35% of the respondents, false positive and negatives were stated as major challenges.

Managers and laboratory technologists' views

Malaria managers commented that RDT readings were problematic – "*the fault could have been due to the clinic staff not reading the test in time.*" One laboratory technologist said that the nurses were not doing the test properly – "*When we receive the test back we see that they are putting too much blood.*"

Discussion

Although this study involved a small representative sample of users of RDT at the primary care facilities in South Africa, it has revealed that (1) there are sporadic problems of stock-out of RDT during peak transmission seasons; (2) the storage facilities and monitoring is inadequate in many primary care facilities; (3) the accuracy of the RDT was tested on an *adhoc* basis leading to nurses sometimes offering antimalarials to RDT-negative cases on the basis of their clinical judgment; (4) there is a possibility that RDTs under-diagnose malaria in the current context and (5) there is very limited training on the use of RDT at the PHC level.

Quality control from nurses' perspectives : selected quotes

- *"our results did not correlate with the patients signs and symptoms"*
- *"our results did not correlate with the lab findings"*
- *"unsure of a negative results"*
- *"we find it positive they (lab) find it negative"*
- *"clinically suspected cases were negative on RDT but positive in the hospital"*

Quality control from the perspective of researchers and malaria control programme managers

- *"Accuracy of the test, we are not getting the required level of sensitivity"*
- *"No commercial control is available"*
- *"we are concerned about the stability of the quality control specimen"*
- *" the key challenges are operator level efficiency, quality of the actual test, training instruction in the tests itself and performance of the test in the field"*
- *"Finding the right specimens and sources of the specimens for quality control, poses a huge challenge"*
- *"it is difficult to try to tease out what the problem really is in doing the test, ensure that the variables are all the same when testing proficiency."*
- *sometimes smears are done in parallel with the RDT and sometimes there is discrepancy."*

Figure 3
Quality-related challenges: selected quotes by nurses, researchers and malaria control managers.

Positive aspects:

- *"It is rapid and easy to use and it can enable treatment and can prevent complication."*
- *"Test is easy to use a nurse can make a diagnosis without involving the doctor and treat the patient".*
- *"The community is aware that such a test exists, they come to clinic and ask for a malaria test."*
- *"We can do the test at the clinic no need to go to the hospital."*
- *"I can praise it because it can save someone's life."*

Figure 4
Nurses quotes on positive aspects of RDT and Summary of negative aspects of the RDTs.

According to the WHO, temperatures above 30°C are considered inappropriate for storing RDTs [4]. As temperatures above 30°C can affect overall performance of the RDTs, temperature-monitoring needs to take place in all clinics and environments. Air-conditioning or similar cooling equipment should be considered in those clinics that exceed the WHO recommended threshold [4]. RDT should be stored in a centralized store as long as possible and care should be taken during transport and storage at the health facilities to minimise degradation. Use of positive control wells and temperature monitors should be considered in South Africa to assure the quality of the RDTs and to build confidence of the users on RDT [9-11].

The quality of the RDT can be established at three levels: post-manufacture level, end user level and through the use of positive control wells [9,12]. Due to the uncertainty of the quality of the test and lack of confidence in some cases of interpreting the results, patients were getting inappropriate treatment. For example some cases were given antimalarial treatment on clinical diagnosis even if the RDT was negative. It is possible to obtain false negative RDT results [6]. However there is no system of evaluating the performance of RDT in the routine health services and to build confidence among the users of RDT. Very few users at the primary health care clinics were formally trained in performing and interpreting RDTs. Thus an in-service training and quality control system is needed urgently to ensure appropriate use of RDTs and effective treatment of malaria in South Africa. Although package inserts are useful it would be easier for the end-user to

have posters or job aids so that the test procedure can be easily visible and read especially during busy periods and late in the night [13-15]. End user proficiency testing such as those described in other studies may be considered[13,14,16].

Conclusion

This study in South Africa may not be applicable to other countries in Africa. However, the lessons on storage, quality assurance and training observed in this study would be applicable to most settings where RDTs are introduced. Further studies of the factors influencing the appropriate use of RDTs and the ways to improve the use of RDTs are needed in the study setting.

Authors' contributions

All the authors conceptualized the study, participated in the analysis, drafting of the manuscript and writing the final version of the paper.

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